National Research Council Canada

Conseil national de recherches Canada

NRC · CNRC

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

ISSN 0706-652X / CJFSDX 53(7) 1473–1694 (1996)

The *Canadian Journal of Fisheries and Aquatic Sciences*, previously published as *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, and *Journal of the Fisheries Research Board of Canada* 1938–1979, is issued monthly to form annual volumes of 12 numbers plus supplements.

Editorial policy: The *Journal* publishes original research articles, critical reviews, perspectives (essays of opinion or hypothesis), and comments. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmation state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

Manuscripts for publication should be submitted to **Dr. D.G. Cook**, Canadian Journal of Fisheries and Aquatic Sciences, NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada (613-993-2209; fax: 613-952-7656; e-mail: journal.cjfas@nrc.ca).

Submissions in either English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. NRC accepts no responsibility for statements or opinions expressed by a contributor. Acceptance of an advertisement, announcement, or other material does no imply endorsement by either the *Journal*'s Editors or NRC. The use of proprietary names does not imply endorsement of the product or company.

Correspondence concerning accepted manuscripts and corrected proofs should be sent to NRC Research Press, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Instructions to Authors are published once a year in the first issue of each volume; copies can be obtained from the Editorial Office, the Publishing Office, or at http://www.nrc.ca/cisti/journals/.

Microfilm: Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, U.S.A.

U.S. periodical postage paid at Champlain, N.Y., and at additional mailing offices. Attention U.S. POSTMASTER: Send address changes to **Canadian Journal of Fisheries and Aquatic Sciences**, c/o USACAN Media Distribution Center, P.O. Box 2888, Plattsburg, NY 12901, U.S.A.

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, et *Journal of the Fisheries Research Board of Canada* 1938–1979. Le *Journal* paraît tous les mois, formant ainsi un volume de 12 numéros, et offre en plus jusqu'à deux suppléments par année.

Politique de rédaction : Le journal publie des articles fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (perspectives) et des commentaires. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influent sur les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverse façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel de connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus de corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donnée) et tenir à une épuration ou une reformulation des connaissances actuelles.

Les auteurs doivent adresser leurs **manuscrits** à **D.G. Cook**, Journal canadien des sciences halieutiques et aquatiques, Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada (613-993-2209); télécopieur : 613-952-7656; courrier électronique : journal.cjfas@nrc.ca).

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à.-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le CNRC ne se tient en aucune façon responsable des opinions exprimées par un collaborateur ou des déclarations qui pourrait faire ce dernier. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par les directeurs du *Journal* ou du CNRC. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

La correspondance ayant trait aux manuscrits acceptés et les **épreuves corrigés** doivent être envoyées aux Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Recommandations aux auteurs sont publiés une fois par année dans le premier numéro de chaque volume. On peut s'en procurer une copie, sur demande, au Bureau de la rédaction, au Bureau des publications et au http://www.nrc.ca/cisti/journals/.

Microfilms : On peut acheter sur microfilms de 16 ou 35 mm tous les numéros depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de University Microfilms International, 330 North Zeeb Road, Ann Arbor, MI 48106, É.-U.

This journal is indexed or abstracted in / Ce journal est signalé ou résumé dans
Aquatic Sciences and Fisheries Abstracts, Biological & Agricultural Index, CAB Abstracts (Centre for Agriculture and Biosciences), Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, GEOBASE – Geo Abstracts, Meteorological and Geoastrophysical Abstracts, Oceanic Abstracts, Science Citation Index, Biological Abstracts, Environment Abstracts, Environmental Periodicals Bibliography, GEOREF, Life Sciences Collection, and PASCAL Database

Volume 53, Number 7, July 1996

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 53, numéro 7, juillet 1996

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

Editor / directeur scientifique D.G. Cook

Assistant to the Editor / adjointe au directeur scientifique **M.M. Milne**

Consulting Editor / rédacteur-conseil **P.E.K. Symons** (Victoria, B.C.)

Consulting Editor / rédactrice-conseil **N. Moir** (Nepean, Ont.)

Editorial Board / conseil des rédaction

S.E. Campana, *DFO/MPO, Dartmouth*
E.M.P. Chadwick, *DFO/MPO, Moncton*
L.M. Dickie, *DFO/MPO, Dartmouth*
G.S. Jamieson, *DFO/MPO, Nanaimo*
J.R.M. Kelso, *DFO/MPO, Sault Ste. Marie*
K.R. Munkittrick, *DoE/MdE, Burlington*
J.D. Neilson, *DFO/MPO, St. Andrews*
L.S. Parsons, *DFO/MPO, Ottawa*
R.M. Peterman, *Simon Fraser University*
R.D. Robarts, *DoE/MdE, Saskatoon*
J.C. Roff, *University of Guelph*
D.M. Rosenberg, *DFO/MPO, Winnipeg*
P. Schwinghamer, *DFO/MPO, St. John's*
(Deputy editor / rédacteur adjoint)
C.J. Walters, *University of British Columbia*
W.G. Warren, *DFO/MPO, St. John's*
N.H.F. Watson, *DFO/MPO, Halifax*

NRC Research Press / Presses scientifiques du CNRC

Editor-in-chief / directeur général **Bruce P. Dancik**

Assistant Editor-in-chief / directeur général adjoint **Iain E.P. Taylor**

Publishing Office / Bureau des publications

(Fax / télécopieur : 613-952-7656; e-mail / courrier électronique : research.journals@nrc.ca;
URL: http://www.nrc.ca/cisti/journals/)

Director / directrice **Aldyth Holmes**

Business Manager / gestionnaire des affaires **Paul McClymont**

Production Manager / gestionnaire de la production **Susan R. Strahlendorf**

Senior Publication Officer / agent principal de publication **Mike Boroczki**

Publication Officers / agentes de publication **Jennifer Douglas (613-993-0368), Jennifer McColl (613-993-4500)**

Publication Officer (French manuscripts) / agente de publication (manuscrits français) **Ginette Fortier (613-993-9117)**

Advertising / annonces publicitaires **Hoda Jabbour (613-993-9085)**

Reprints / tirés à part **Judy Heyman (613-993-0151)**

Subscriptions / abonnements **Helen Goulet (613-993-9084)**

© National Research Council of Canada 1996. World rights reserved.

No part of this publication may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher, except as stated below.

The National Research Council of Canada grants **permission*** to individuals to make single copies of articles for personal use. Any person may reproduce short excerpts from articles in the journal for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal use, as stated above, is granted by the National Research Council of Canada provided that the copyright fee of $3.00 per copy is paid directly to the Copyright Clearance Center (CCC) Transactional Reporting Service, 21 Congress Street, Salem, MA 01970, U.S.A. 0706-652x/96 $3.00 + 0.00.

*The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

© Conseil national de recherches du Canada 1996. Tous droits réservés.

Il est interdit de reproduire, de sauvegarder ou de transmettre quelque partie de la présente publication dans quelque format ou de quelque façon sans le consentement écrit de l'éditeur, à l'exception des conditions suivantes.

Le Conseil national de recherches du Canada accorde aux particuliers le droit de faire des copies individuelles pour leur usage personnel. Toute personne peut reproduire de courts extraits des articles de la Revue pour autant que les droits moraux de l'auteur soient respectés et que la mention de provenance soit clairement indiquée. Il est de convenance de demander le consentement de l'auteur avant de faire mention de son travail.

On obtiendra l'autorisation de reproduire des articles à d'autres fins que l'usage personnel (tel qu'on l'a décrit ci-dessus) auprès du Conseil national de recherches du Canada pour autant que des redevances de droits d'auteurs de 3,00 $/copie soient versées au Copyright Clearance Center (CCC) Transactional Reporting Service, 21 Congress Street, Salem, MA 01970, U.S.A. 0706-652x/96 3,00 $ + 0,00.

*La permission ci-dessus ne s'applique pas à la reproduction à grande échelle, telle que la reproduction aux fins de distribution générale, publicitaire ou promotionnelle, de compilation de nouveaux ouvrages collectifs ou de revente. Dans de tels cas, il faudra ratifier une entente particulière avec l'éditeur, le Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Subscription information

The *Canadian Journal of Fisheries and Aquatic Sciences* (ISSN 0706-652X) is published monthly by the National Research Council of Canada. 1997 annual subscription rates (second class postage) for Canada are $461CAN institutional and $157CAN personal, for US & foreign $461US institutional and $167US personal. For air mail add $150US to foreign rate. Canadian customers please include 7% GST (GST no. R121491807). In Quebec please add a further 6.5% QST to the total price including GST (QST no. 1006178088). Subscriptions are entered for the calendar year. Remittances should be made payable to the Receiver General for Canada credit NRC. Price for regular single issues: institutional, $60.00; personal, $25.00. Supplements will be priced separately. Special rates apply to members of Canadian scientific and engineering societies if they subscribe through their respective societies.

There are no refunds on cancelled subscriptions once delivery has begun. Claims for missing issues should be made within four months (N. America) or six months (outside N. America) following the date of publication. The publisher expects to supply missing issues only when losses have occurred in transit and when the reserve stock will permit. For change of address and subscription enquiries: Subscription Office, NRC Research Press, National Research Council Canada, Ottawa, ON K1A 0R6, Canada; tel: 613-993-9084; fax: 613-952-7656; e-mail: research.journals@nrc.ca.

Microform backfiles are available from UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, U.S.A., and Micromedia Ltd., 20 Victoria St., Toronto, ON M5C 2N8, Canada.

Renseignements sur l'abonnement

Journal canadien des sciences halieutiques et aquatiques (ISSN 0706-652X) est une publication mensuelle du Conseil national de recherches du Canada. Le prix de l'abonnement annuel au Canada (courrier de deuxième classe) est de 461 $CAN pour les organismes et de 157 $CAN pour les particuliers. À l'étranger (y compris les É.-U.), le prix de l'abonnement est de 461 $US pour les organismes et de 167 $US pour les particuliers. L'expédition par avion exige des frais de 150 $US en sus du prix d'abonnement à l'étranger. Les clients du Canada doivent ajouter la TPS de 7% (nº de TPS R121491807). Au Québec, il faut ajouter la TVQ de 6,5% au prix total, TPS comprise (nº de TVQ 1006178088). Les abonnements sont pour l'année civile. Tout paiement doit être adressé au Receveur général du Canada, crédit du CNRC. Le prix par numéro est de 60 $ pour les organismes et de 25 $ pour les particuliers. Le prix des hors-série varie. Les membres des sociétés canadiennes de sciences et de génie bénéficient de taux préférentiels en s'abonnant via leurs sociétés respectives.

Aucun remboursement ne sera accordé ni aucune annulation acceptée une fois que les livraisons auront commencé. Les réclamations de numéros manquants devront être soumises dans les quatre mois (Amérique du Nord) ou dans les six mois (à l'extérieur de l'Amérique du Nord) suivant la date de publication. L'éditeur ne fournira de numéros manquants que si le document a été perdu pendant l'expédition et que si le stock le permet. Adressez tout changement d'adresse ou toute question quant à l'abonnement au : Bureau des abonnements, Presses scientifiques du CNRC, Conseil national de recherches Canada, Ottawa, ON K1A 0R6, Canada; téléphone : 613-993-9084; télécopieur : 613-952-7656; courrier électronique : research.journals@nrc.ca.

On peut obtenir les fichiers rétrospectifs sur **microforme** auprès de UMI, P.O. Box 1346, Ann Arbor, MI 48106-1346, É.-U., et auprès de Micromedia Ltée, 20 rue Victoria, Toronto, ON M5C 2N8, Canada.

Recent excursions of capelin (*Mallotus villosus*) to the Scotian Shelf and Flemish Cap during anomalous hydrographic conditions

K.T. Frank, J.E. Carscadden, and J.E. Simon

Abstract: Episodic occurrences of capelin (*Mallotus villosus*) outside their normal range were evident from examination of current and historical records of the Scotian Shelf and Flemish Cap. Capelin reappeared in both locales, beginning in the late 1980s (Scotian Shelf) and early 1990s (Flemish Cap), concomitant with anomalous meteorological and hydrographic conditions and an abrupt decline in offshore abundance in their principal centre of distribution (division 2J3KL). Movement of capelin to these regions is associated with lower than normal ocean temperatures but not with warmer than normal conditions. In both locales adult capelin were the first to appear, and spawning was evident; successful reproduction has occurred on the Scotian Shelf (a 1993 year-class formed). The increase in capelin abundance was not limited to the eastern Scotian Shelf and Flemish Cap, and capelin was not the only species involved. Capelin in the southern Gulf of St. Lawrence exhibited a very similar temporal pattern of abundance, and two Arctic–boreal species (Greenland halibut (*Reinhardtius hippoglossoides*) and checker eelpout (*Lycodes vahlii*)) exhibited spatial and temporal patterns of abundance similar to those of capelin on the Scotian Shelf. We suggest that capelin may serve as an instructive analog for assessment of the relative influences of environmental effects and fishing on heavily exploited species.

Résumé : L'occurrence épisodique du capelan (*Mallotus villosus*) à l'extérieur de son aire normale est apparue évidente à l'examen des données actuelles et historiques recueillies sur la plate-forme néo-écossaise et le Bonnet flamand. Le capelan est réapparu dans ces deux régions à partir de la fin des années 1980 (plate-forme néo-écossaise) et du début des années 1990 (Bonnet flamand), ce qui correspondait à des conditions météorologiques et hydrographiques inhabituelles et à une baisse brutale de l'abondance de ce poisson plus au large, dans sa zone principale de répartition (division 2J3KL). Le déplacement du capelan vers ces régions est associé aux températures de l'océan inférieures à la normale mais non aux températures supérieures à la normale. Dans les deux régions, les adultes étaient les premiers à apparaître, et l'activité de fraye était évidente; la reproduction a réussi sur la plate-forme néo-écossaise (constitution d'une classe de 1993). La hausse de l'abondance du capelan ne s'est pas limitée à l'est de la plate-forme néo-écossaise et au Bonnet flamand, et le capelan n'a pas été la seule espèce à connaître une hausse. Le capelan du sud du golfe du Saint-Laurent a connu un régime temporel d'abondance très comparable, et deux espèces arctiques–boréales (flétan noir (*Reinhardtius hippoglossoides*) et lycode à carreaux (*Lycodes vahlii*)) présentaient des régimes spatiaux et temporels d'abondance similaires à ceux du capelan sur la plate-forme néo-écossaise. Nous pensons que le capelan peut servir d'exemple instructif pour l'évaluation des influences relatives des conditions environnementales et de la pêche sur les espèces fortement exploitées.
[Traduit par la Rédaction]

Introduction

Distinguishing between the effects of fishing and environmentally induced changes on stock distribution and production has proved to be an important but very difficult task. This has stimulated the analysis (and development) of historical data sets during times when there was no fishing (e.g., Soutar and Isaacs 1974) or when the effects of fishing were slight (Anonymous 1995). Some notable events in the commercial fisheries have occurred during the past century in the Northwest Atlantic, suggesting an influence of anomalous ocean climate conditions. During the winter of 1877–1878, unusually large schools of Atlantic cod (*Gadus morhua*) apparently emigrated a distance of nearly 1000 km from the Grand Banks (division 3NO) to the shores of the New England coast (Earll 1880). This migration may be related to anomalous oceanographic conditions, because at about the same time (1882), enormous numbers of tilefish (*Lopholatilus chameleonticeps*) succumbed to the onset of sudden cold-water conditions occurring in the mid-Atlantic Bight (Cushing 1982), hypothesized to have resulted from an increased transport of the Labrador Current (Anonymous 1995). More recently, the collapse of the northern cod stock in NAFO division 2J3KL (Fig. 1) has coincided with large-scale changes in distribution towards deeper and more southerly portions of the stock range. deYoung and Rose (1993) speculated that the recent changes in cod distribution were due to widespread, below-normal water temperatures and that the distributional changes have in turn resulted in poor recruitment. On the other hand, Hutchings and Myers (1994) rejected hypotheses that attribute the col-

Received December 5, 1994. Accepted February 15, 1996.
J12658

K.T. Frank[1] **and J.E. Simon.** Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, Dartmouth, NS B2Y 4A2, Canada.
J.E. Carscadden. Department of Fisheries and Oceans, Science Branch, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, NF A1C 5X1, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: k_frank@bionet.bio.ns.ca

© 1996 NRC Canada

Fig. 1. Map of the Northwest Atlantic showing NAFO subareas and divisions.

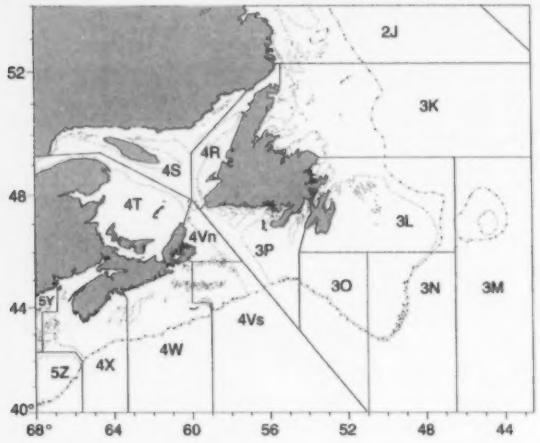
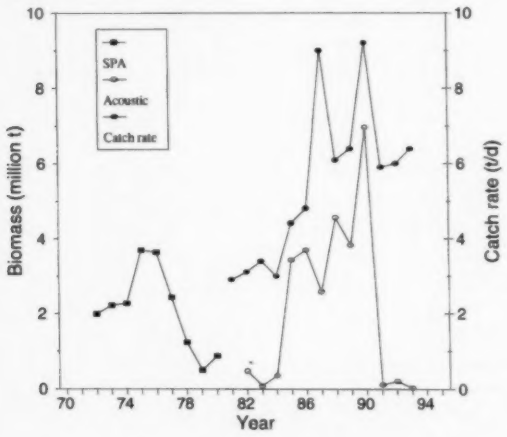


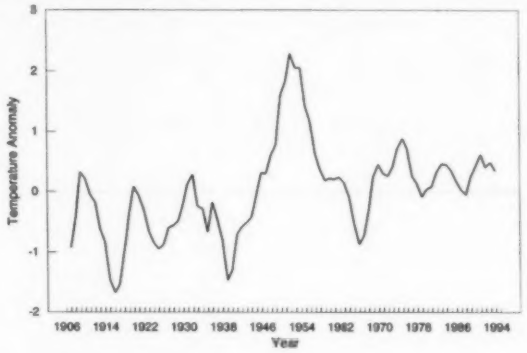
Fig. 2. Trends in abundance of division 3L capelin from 1974 to present. Note dramatic decline in acoustic estimates after 1991 compared with the near-average catch rate values. SPA, sequential population analysis (see Carscadden and Miller 1981, Table 7). Acoustic estimates for division 3L, spring 1982–1992 and fall 1993 are from Miller (1992, 1993); catch rates are from Nakashima (1994).



lapse of northern cod to environmental change and concluded that the collapse can be attributed solely to overexploitation. This ongoing debate underscores the difficulty of unambiguously demonstrating the relative influences of environmental effects and fishing on heavily exploited species. While the ultimate cause(s) of the northern cod collapse may never be fully resolved, we feel that an instructive analog may exist for interpreting the recent past events in the Northwest Atlantic ecosystem, i.e., the capelin (*Mallotus villosus*). It has an identical prinicipal centre of distribution (division 2J3KL), abundance estimates have declined dramatically in the offshore waters, and in contrast to cod it is only lightly exploited.

Capelin is a major diet item of most commercially important groundfish species, and its principal centre of distribution coincides with division 2J3KL (see Akenhead et al. 1982). Since 1991, capelin spawning time has been delayed by up to 1 month (Carscadden et al. 1996), and there has been a shift in distribution from division 2J to areas further south (Lilly and Davis 1993; Miller 1994), similar to groundfish distribution shifts. In addition, traditional abundance indices that, for several years, showed agreement are now widely divergent. Offshore acoustic estimates, which measure recruitment, declined abruptly and have remained low, while inshore indices, which monitor mature fish, have remained at about the same level (Fig. 2). Capelin abundance was relatively high in the mid-1970s, low in the late 1970s and early 1980s, and high during the mid-1980s. Finally, capelin can be considered lightly exploited in the Northwest Atlantic. Shelton et al. (1993) estimated that catches in division 3L during 1982–1989 were 4.3% of the mature biomass and 10% or less in the division 2J3K management unit. (Note that, as of 1994, the current management unit is division 2K3KL.)

Fig. 3. Annual mean sea surface temperature anomalies from Boothbay Harbor, Maine. Long-term annual mean temperature calculated for the period 1906–1990. This data series is generally representative of the low-frequency, large-scale temperature trends across the Gulf of Maine and southwestern Scotian Shelf (Petrie and Drinkwater 1993). The data were smoothed using a 3-year, centre-weighted running mean.

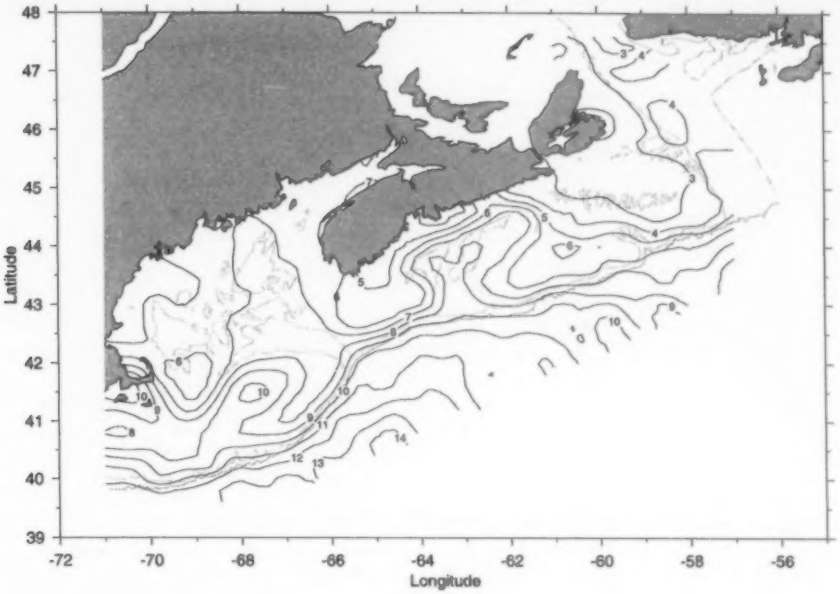


In the fisheries literature, capelin have occasionally been reported to occur in areas outside of their normal distributional range. For example, between 1965 and 1968, unusually high numbers of capelin were recorded in the Bay of Fundy herring weir fishery, and it was suggested that they were reproducing successfully there (Tibbo and Humphreys 1966; Jangaard 1974). This occurrence of capelin in the Bay of Fundy coincided with lower than normal ocean temperatures associated with a cooling trend from 1952 to 1967 (Fig. 3). Colton (1972) suggested that this cooling was a possible cause for the southward extension of capelin. In addition to the 1960s, capelin were reported in the Bay of Fundy between 1915 and 1919 (Bigelow and Schroeder 1953) and around 1903 (Huntsman 1922). Both of these observations of capelin were associated with periods of below-normal water temperatures (Fig. 3).

In more northern waters in the Northwest Atlantic, capelin have extended their range either during warming periods or through changes in the areal extent of the Labrador Current. Dunbar (1966) suggested that the occurrence of capelin in

© 1996 NRC Canada

Fig. 4. Distribution of annual average bottom temperatures on the Scotian Shelf and Gulf of Maine based on all available data.



Ungava Bay in 1884 and 1959 indicated warming conditions. It is interesting to note that the 1884 occurrence of capelin in Ungava Bay has been linked to a warming period in that area but is about the same time that cod moved from division 3NO to New England and significant mortalities of tilefish occurred, ostensibly because of unusually cold conditions. Templeman (1976) concluded that an unusual occurrence of capelin on the Flemish Cap in 1973 was associated with displacement of the Labrador Current towards the Cap. The geographic extent of capelin distributions are apparently influenced by the prevailing oceanographic conditions with occurrences during the past century reported in areas to the north, south, and east of their principal centre of distribution.

It appears that we are now witnessing a concurrent southward and eastward extension of capelin distribution, particularly on the eastern Scotian Shelf (division 4VW) and Flemish Cap (division 3M), respectively (Fig. 1). These events coincide with the occurrence of atmospheric and oceanic extremes in the Labrador Sea – Newfoundland Shelf region and the Gulf of St. Lawrence. In general, lower than normal air and water temperatures and severe ice conditions, in terms of duration and areal extent, have prevailed in these regions during the past 4–6 years (Drinkwater et al. 1995). In this paper, we document these recent unusual occurrences of capelin on the eastern Scotian Shelf and Flemish Cap in the context of their historical occurrences in these areas and in relation to recent hydrographic events.

Materials and methods

Data sources, detailed below, differed in the two study areas. In the Scotian Shelf area, biological and hydrographic data from research vessel surveys were the major sources of data. Other information came from records of by-catch in the commercial fisheries.

For the Flemish Cap, biological data existed from historical research vessel surveys and from recent Spanish bottom trawl research surveys. Hydrographic data were available from research surveys. Historical by-catch data were available as well as detailed by-catch data from a new shrimp fishery that developed in 1993. Other information on the occurrence of capelin on the Flemish Cap was available from historical predator feeding studies and ichthyoplankton surveys.

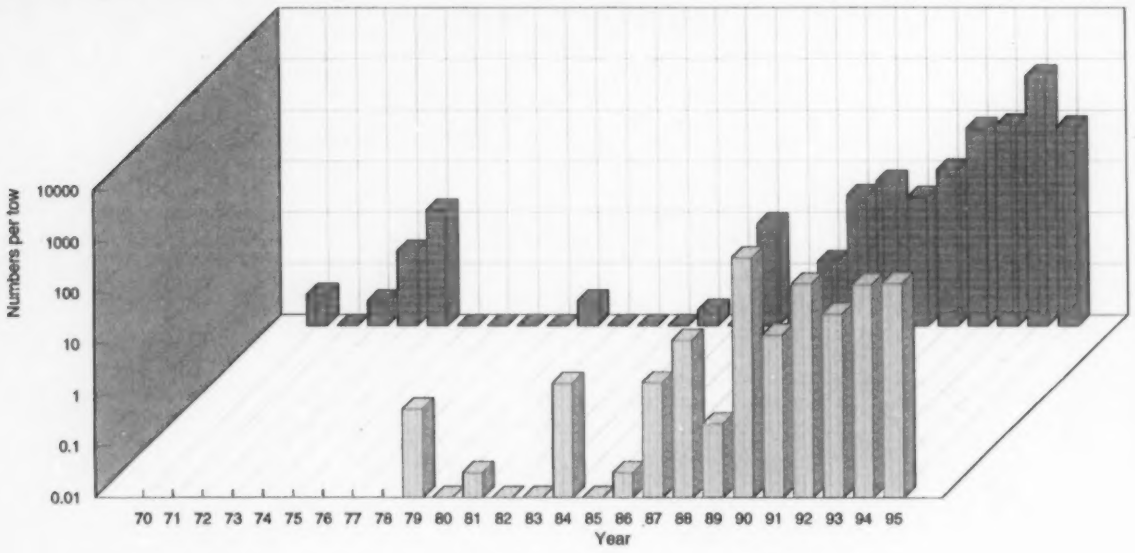
Scotian Shelf

Study area

The Scotian Shelf lies offshore of Nova Scotia and has a length of 700 km and a width, defined by the 200-m isobath, varying from 250 km at its eastern end to 150 km to the west (Fig. 4). The complex topography includes several deep basins (>200 m) near midshelf and a series of shallow (<50 m) banks further offshore. In general the lowest bottom water temperatures are found in the northeast and the warmest in the deep basins, particularly in the central region, and along the shelf break (Fig. 4). The Scotian Shelf is separated bathymetrically from the southern Newfoundland shelf by the Laurentian Channel and from the Gulf of Maine by the Northeast Channel. Stratification in summer in the western region consists of three layers: a near-surface layer with salinities <32‰ and temperatures >5°C, a cold middle layer with salinities 32–33.5‰ and temperatures <5°C, and a warm (>5°C), saline (>33.5‰) bottom layer. In winter the top two layers coalesce. In the eastern Scotian Shelf, there are only two layers in summer as the cold middle layer extends to the bottom. From the standpoint of habitat suitability for capelin, only the northeastern sector of the Scotian Shelf would qualify as suitable; all other areas on the shelf and Gulf of Maine regions are too warm, on average, for capelin to inhabit (Fig. 4). We based this conclusion on observations by Pitt (1958) and the more extensive data presented in Carscadden et al. (1989). They showed that successful reproduction and development in offshore areas, such as the southern Grand Banks (division 3NO), was associated with bottom water temperatures in the 2–4°C range. However, temperature is not the only condition necessary for successful reproduction; factors such as bottom type, water depth, and a hydrographic containment system are also important.

© 1996 NRC Canada

Fig. 5. Mean numbers of capelin per tow for division 4VW during spring (1979–1995) and summer (1970–1995) RV surveys. Note that these catch rates represent only the eastern half of the Scotian Shelf because generally no capelin were collected in division 4X.



Research vessel surveys

Research vessel (RV) surveys of the Scotian Shelf have been conducted each spring since 1979 (NAFO division 4VsW) and each summer since 1970 (NAFO division 4VWX) by the Canadian Department of Fisheries and Oceans (DFO). Both sampling and data analysis protocols are well documented (Halliday and Koeller 1981; Smith and Somerton 1981). Because the cod end of the western II-A bottom trawl is equipped with a 19-mm liner, by-catch of juvenile groundfish, pelagic fish, and small-bodied fish species in general is common. Each trawl sample (referred to as a set) is randomly selected within strata that are defined on the basis of depth and allocated in proportion to stratum area. Set totals (up to and including 1995) amount to 2907 and 4018 for the spring and summer surveys, respectively.

Biological sampling of capelin included estimation of size (length frequency), total weight, and numbers in each tow. Capelin distributions were generated from individual sets and plotted using ACON version 7.27 (Black 1991). For each annual survey, capelin abundance is expressed as stratified mean numbers per tow by division.

By-catch in commercial fisheries

There is no directed fishery for capelin on the Scotian Shelf, but we examined NAFO catch statistics (1961–1988) and zonal catch records (1989–present) for reports of capelin catches, which would have been reported as by-catches in other fisheries.

Hydrographic data

Hydrographic data were collected at the end of each trawling station during the RV surveys. Before 1990, water temperature and salinity data were obtained from water samples collected with a reversing bottle from near bottom. Water temperatures were measured in 1990–1995 using an internally recording Seabird model 19 (or 25) conductivity, temperature, and depth (CTD) profiler. The near-bottom temperatures from each CTD profile were calibrated with the reversing thermometer estimates of temperature (Page and Losier 1994).

Flemish Cap

Study area

The Flemish Cap (NAFO division 3M) is a relatively small bank (about 52 × 42 nautical miles (1 nautical mile = 1.853 km) bounded by the 200-m isobath) centred at about 47°N, 45°W (Fig. 1). It is separated from the northern Grand Banks to the west by the Flemish Channel (Pass), which is over 1000 m deep (Templeman 1976). Flemish Cap is influenced by two major current systems. Cold, low-salinity water from the Labrador Current flows from the west and splits into two branches at the northwestern corner of Flemish Cap. A southward flowing branch moves through the Flemish Pass, between the Grand Bank and the Flemish Cap, while the other branch flows east and then southeast around the northern and northeastern slopes of the Flemish Cap. The North Atlantic Current, warmer and more saline than the Labrador Current, flows east–northeast across the southern slopes of the Flemish Cap. There is a weak anticyclonic gyre over the central portion of the Flemish Cap (Lilly 1987). Mean monthly bottom (100–150 m) water tempeatures on the central portion of the Flemish Cap range between 3 and 4°C, and at similar depths in the western slope region of the Cap, monthly temperatures are nearly identical (Drinkwater and Trites 1986). These temperatures would not be limiting to capelin spawning (Carscadden et al. 1989).

Research vessel surveys

The Flemish Cap has supported major fisheries for cod and redfish (*Sebastes* spp.) (Templeman 1976; Lilly 1987), and as a result it has been the site of RV surveys aimed at groundfish since the 1940s. Because of its geographic location, this area is in international waters and several countries have conducted these surveys. We review the results of these surveys as well as ichthyoplankton surveys in the area. In addition, we present capelin by-catch data from Spanish surveys conducted since 1988. The Spanish surveys have used a stratified random design (stratified by depth) with tow depths down to 720 m during late June to early August each year. Each survey has in excess of 100 (106–117) valid tows. The gear has remained unchanged and the trawl cod end has a 35-mm mesh liner (Vasquez 1993). Numbers of capelin per tow and lengths of individual capelin were recorded.

© 1996 NRC Canada

Distributions of capelin from the surveys were plotted using ACON version 7.27 (Black 1991). Tow duration was usually 30 min, but because some tows deviated from this, we expressed capelin abundance as catch in numbers per hour.

By-catch in commercial fisheries

Similar to the Scotian Shelf, there have been no directed fisheries for capelin, but NAFO catch statistics were examined for reports of capelin by-catch.

During April 1993, a shrimp fishery developed on the Flemish Cap. Data for individual tows collected on Canadian shrimp vessels during May, June, and July by fisheries observers included date, time, location, duration, depth of tow, and by-catch weight by species. Capelin occurred in the by-catch and samples were frozen for later detailed biological sampling in the laboratory. At that time, data such as length, sex, maturity, and ovary weight were collected.

Hydrographic data

Hydrographic data have been collected during RV surveys by the DFO for several years. Data collected during July 1993 were recently analyzed in relation to the available historical time series of temperature and salinity for the Flemish Cap area (Colbourne 1993).

Results

Scotian Shelf

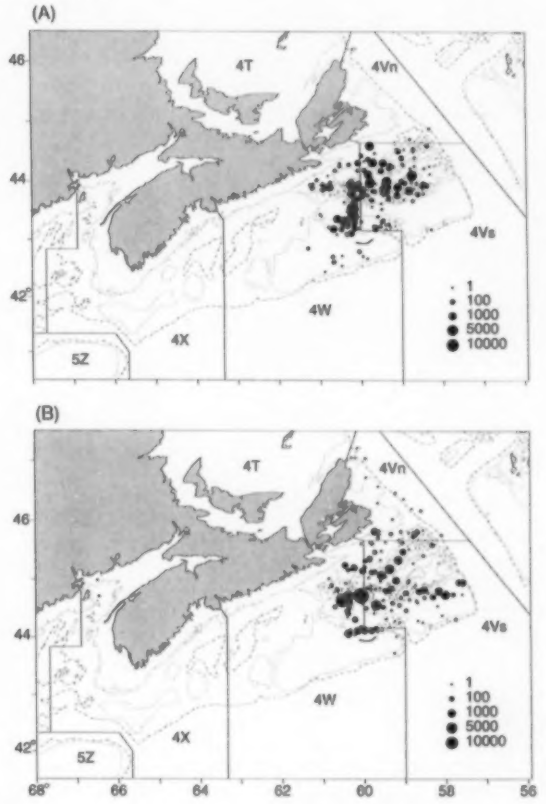
Research vessel surveys

The spring and summer RV surveys both showed a progressive increase in capelin abundance in division 4VW since 1987 (Fig. 5) with the catch rate during the 1994 summer survey being the highest ever observed. Throughout the remainder of the survey series, capelin were not abundant except for the early 1970s when catch rates exhibited a minor peak that ended abruptly in 1975 (Fig. 5).

This recent episode of increased capelin abundance appeared to be confined to the offshore waters of the eastern Scotian Shelf, as revealed by the spring and summer RV surveys (Fig. 6). Very few capelin were encountered in the Bay of Fundy during the summer surveys, and there have been no reports of capelin occurring in the herring weir fishery after 1968 (W. Dougherty and C. Monaghan, DFO port technicians, Biological Station, St. Andrews, NB E0G 2X0, personal communication). It appears, therefore, that the present capelin incursion is not as extensive as in the past (e.g., 1960s as described by Tibbo and Humphreys 1966), with the leading edge of the current distribution occurring near the boundary between divisions 4W and 4V. Almost no capelin were encountered west of 61°W longitude (Fig. 6).

The size frequency distribution of capelin in division 4VW revealed the initial presence of large individuals followed by a progressive representation of smaller fish (Table 1). This pattern was evident in the mid-1970s and more recently in 1987 in both the spring and summer surveys. Since the incursion in the late 1980s the size distribution of the adult population has steadily increased with gradual accumulation of smaller individuals through recruitment (Table 1). The mode at 6 cm in the 1994 spring RV is strong evidence for local production of a year-class in 1993 (Table 1). Although none of the fish was aged it is known that capelin >14 cm are 2+ years old. This is based on age readings conducted by

Fig. 6. Geographic distribution of capelin on the Scotian Shelf depicted as expanding symbols of capelin catch per tow from the spring (A) and summer (B) RV surveys for all years combined. Note that the spring RV survey did not include division 4X or division 4Vn.



Tibbo and Humphreys (1966) of capelin from the Bay of Fundy and capelin in other areas (Winters 1982). Given the spread in the size frequencies recorded it is obvious that several age groups were co-occurring in this geographic area, suggestive of a self-sustaining population.

By-catch in commercial fisheries

There were no capelin catches reported for division 4VWX between 1989 and 1994. However, 37 and 124 t of capelin were reported caught in division 4X (includes Bay of Fundy) in 1965 and 1967. Catches of 1–2 t of capelin were reported from division 4Vn in 1975, 1983, and 1984. The timing of these by-catches was similar to the minor peaks in capelin by-catch in the spring and summer RV surveys on the Scotian Shelf (Fig. 5). On the basis of the recent RV survey results, the DFO granted experimental fishing licenses for capelin in 1994 and 1995. No capelin were landed; however, the areas searched did not coincide with the centres of concentration reported from the RV surveys.

Hydrographic conditions

Coinciding with previous southward extensions of capelin into

© 1996 NRC Canada

Table 1. Length–frequency distribution of capelin from the spring and summer research vessel surveys of the Scotian Shelf.

Length (mm)	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986
													Spring				
5										0	0	0	0	0	0	0	0
6										0	0	0	0	0	0	0	0
7										0	0	0	0	0	0	0	0
8										0	0	0	0	0	0	0	0
9										0	0	0	0	0	0	0	0
10										0	0	0	0	0	0	0	0
11										0	0	0	0	0	0	0	0
12										1	0	0	0	0	0	0	0
13										6	0	1	0	2	1	0	0
14										5	0	1	0	0	29	0	1
15										9	0	0	0	0	39	0	0
16										1	0	0	0	0	16	0	0
17										0	0	0	0	0	2	0	0
18										0	0	0	0	0	0	0	0
19										0	0	0	0	0	0	0	0
20										0	0	0	0	0	0	0	0
21										0	0	0	0	0	0	0	0
22										0	0	0	0	0	0	0	0
													Summer				
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	4	0
13	0	0	0	5	11	0	0	0	0	0	0	0	0	0	0	16	0
14	0	0	1	11	8	0	0	0	0	1	0	0	0	0	0	8	2
15	1	1	0	8	1	0	0	0	0	0	0	0	0	1	0	3	0
16	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
17	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Note: Numbers were adjusted for subsampling and tow duration. Note that there were no spring surveys during 1970–1978.

Nova Scotian waters, recent water temperatures have been below normal and near-record low levels. Temperature conditions were evaluated from the July RV surveys for the division 4VW region only and within this region the data were aggregated on the basis of the geographic distribution of capelin, i.e., east and west of 61°W longitude (Fig. 6). East of 61°W, where recent capelin catch rates are highest, near-bottom water temperatures have been declining since 1985, with median temperatures generally less than 3°C during 1989 and 1995 (Fig. 7). An opposite temperature trend was evident west of 61°W with near-bottom water temperatures rising since 1987 from 5°C to greater than 8°C in 1995 (Fig. 7). Whenever median temperatures fell below 3°C, capelin appeared as by-catch in the RV survey, particularly during the early 1970s and since 1987. As expected, the correlation between the median near-bottom temperatures east of 61°W (Fig. 7) and capelin abundance (Fig. 5) was highly significant ($r = -0.49$, $n = 26$, $p < 0.01$) and nonsignificant for temperatures west of 61°W ($r = 0.02$). We also evaluated the correlation between bottom temperatures and capelin abundance on a set by set basis for each summer survey. Correlations were highly significant between 1991 and 1995 (range of r –0.25 to –0.41, $p < 0.01$); for 1974 and 1990 the correlations were significant at $p < 0.05$, and for the remaining years the correlations were not significant. Therefore, the abundance and local distribution of capelin on the eastern Scotian Shelf is associated with relatively cold-water conditions. Their absence in other areas on the shelf (i.e., west of 61°W longitude) can be ascribed to water conditions that are too warm.

In summary, we have shown that capelin were generally rare on the Scotian Shelf until recently, that capelin occur as by-catch in the RV survey in this area during colder than normal bottom water conditions, that adult capelin are the first to appear, and that if the cold conditions persist, then successful reproduction occurs. The latest event on the eastern Scotian Shelf has evolved over an 8-year period. We now turn to the

© 1996 NRC Canada

1987	1988	1989	1990	1991	1992	1993	1994	1995
0	0	0	0	0	0	0	5	0
0	0	0	0	0	0	0	2 926	0
0	0	0	0	1	1	0	1 422	0
0	0	0	0	4	0	0	13	0
0	0	0	1	15	0	0	0	9
0	1	0	13	20	3	6	4	144
0	13	0	91	21	11	17	133	621
0	32	0	1 577	36	356	56	409	2 730
2	52	1	6 446	40	1 934	195	860	4 154
13	68	1	8 264	96	3 955	380	773	4 105
19	61	2	7 420	315	5 241	705	339	1 682
25	23	9	2 157	542	3 017	604	132	507
14	1	19	137	154	631	332	104	172
0	0	1	47	2	56	52	37	24
0	0	0	0	3	0	6	6	4
0	0	0	0	0	0	2	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	6	0	0
0	0	0	0	0	0	12	0	2
0	0	0	0	0	8	139	8	52
0	1	0	0	32	22	398	181	511
0	1	3	1	754	54	1 227	362	1 202
0	1	6	3	1 787	48	1 649	346	1 058
0	1	26	26	959	37	459	282	224
0	9	76	69	23	145	436	2 018	1 445
3	156	77	134	12	763	1 208	12 540	2 335
4	401	38	98	21	4 350	1 130	12 925	1 081
10	90	67	40	37	11 399	1 099	6 631	616
1	5	38	7	21	5 329	560	7 780	102
0	0	1	4	1	244	111	2 524	7
0	0	0	3	0	0	7	0	0
0	0	0	3	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

Flemish Cap and assess the recent excursions there relative to the patterns witnessed on the Scotian Shelf.

Flemish Cap

Research vessel surveys

Canadian and USSR bottom trawl RV surveys conducted between 1949 and 1985 reported no capelin by-catches. This is not a result of using inappropriate gear because the same gear used in other areas successfully caught capelin (USSR: Borovkov et al. 1989; Canada: Lilly and Davis 1993 and other records available at DFO, St. John's, Nfld.). Spanish bottom trawl surveys initiated in 1988 did not catch capelin during 1988–1991 but in 1992 and 1993, 74 and 447 individuals, respectively, were caught. In 1992 and 1993, capelin occurred over the north-central part of Flemish Cap with most catches occurring in water depths less than 300 m and fewer catches in the 300–600 m depth range (Fig. 8). We conclude that capelin are rare inhabitants of Flemish Cap and in this respect the situation is similar to the Scotian Shelf.

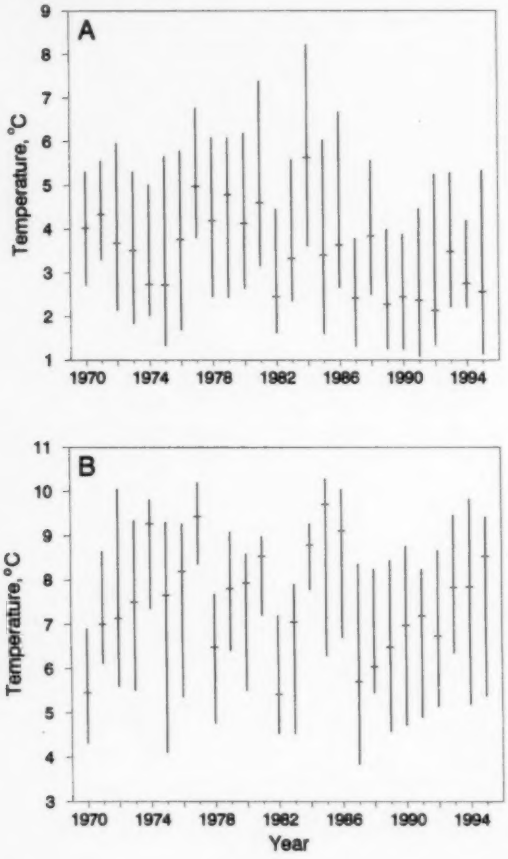
Recent catches of capelin on Flemish Cap were comprised mainly of larger fish, a result similar to the finding for capelin initially caught on the Scotian Shelf in 1987. Length frequencies from the 1992 and 1993 Spanish surveys were similar, with 94% in 1992 and 92% in 1993 in the 15–18 cm length ranges. The similarities in the length composition over these 2 years suggest that different year-classes were recruiting into the population in the Flemish Cap area.

By-catch in commercial fisheries

By-catches of capelin in the Flemish Cap, as reported to NAFO, are unusual. In 1973, 317 t of capelin was reported as by-catch in the redfish fishery. Templeman (1976) attributed this particular occurrence of capelin to unusually cold water on the Flemish Cap that year. No other catches of capelin were

© 1996 NRC Canada

Fig. 7. Annual, near-bottom temperatures from the summer RV survey east of 61°W longitude (A) and west of 61°W (B). Horizontal bar intersecting each vertical line shows median and interquartile range, respectively.

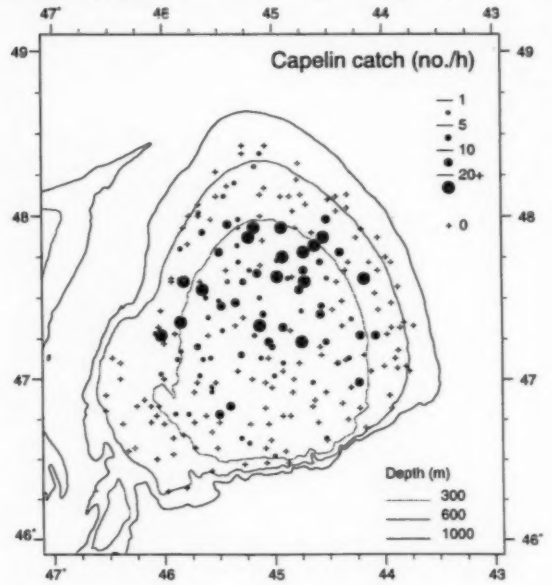


reported until 1990, 1991, and 1992 when 14, 6, and 6 t, respectively, were reported.

During the shrimp fishery on the Flemish Cap, which began in 1993, capelin occurred in 173 (95%) of the 183 observed sets in May (total weight 1052 kg), 485 (65%) of the 742 observed sets in June (total weight 3272 kg), and 137 (77%) of the 178 observed sets in July (total weight 487 kg). The shrimp fishery was conducted primarily in the 300–400 m depth zone and capelin catches occurred throughout the shrimp fishery (Fig. 9). In both May and July, capelin were distributed along the western and northwestern edge of the Flemish Cap spanning over 1° of latitude (approximately 110 km). In June, the distribution was more extensive and dome shaped, covering the western, northern, and eastern flanks of the Flemish Cap. Most of the catches were less than 1 kg/h. While capelin were found consistently wherever shrimp-directed tows were made it is possible that capelin could have been present in other locations and in larger aggregations in the Flemish Cap region.

Length frequencies were similar in all months with most

Fig. 8. Geographic distribution of capelin depicted as expanding symbols of catch per tow in division 3M from RV surveys conducted by Spain in 1992 and 1993 (plotted together).



fish >14 cm. Capelin of age 4 comprised over 80% of the samples with the remainder being ages 3 and 5. The length-frequency distribution was similar to the distribution from the Spanish data with 94% of capelin in the 15–18 cm range.

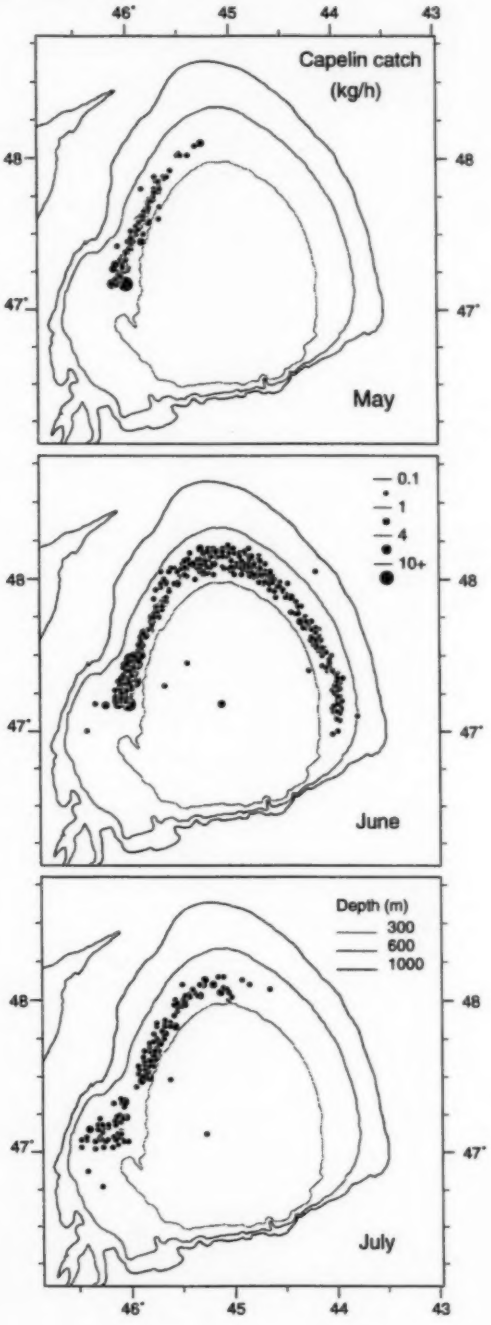
We also compared roe content (weight of roe expressed as a percentage of total body weight) of Flemish Cap capelin with roe content of capelin collected between March and July on the Grand Banks and on the spawning grounds of the Southeast Shoal (Fig. 10). This comparison showed that all gonads were developing at the same rate. The roe content of capelin at about day 180 and later was 25% or greater and represents fish in spawning condition. Like the recent situation on the Scotian Shelf, capelin may spawn on the Flemish Cap.

Other information

From our examination of the literature, we estimate that in excess of 25 000 cod stomachs from the Flemish Cap have been examined between 1978 and 1990 by Canadian, Soviet, and Spanish scientists (Turuk and Postolaky 1980; Turuk 1981; Konstantinov et al. 1985; Albikovskaya and Gerasimova 1989; Paz et al. 1989, 1991; Casas and Paz 1994). These studies cover most months (although not in one year), yet no capelin have been recorded as prey. A similar absence of capelin in the diet has been reported from examinations of over 2000 American plaice (*Hippoglossoides platessoides*) stomachs. In contrast, capelin have been recorded as prey of redfish and Greenland halibut (*Reinhardtius hippoglossoides*), although such records are uncommon. Noskov et al. (1963) reported that in summer 1962, concentrations of redfish were found on the northwestern and northeastern slopes of the Flemish Cap between 300 and 450 m. These redfish were reportedly feeding heavily on amphipods, shrimp, capelin, and lantern anchovy, although quantitative estimates of stomach

© 1996 NRC Canada

Fig. 9. Geographic distribution of capelin during May, June, and July based on incidental catches from the shrimp fishery in division 3M during 1993.



contents were not provided. In a study of feeding by Greenland halibut in Flemish Pass in 1992, capelin were found to be rare (<1% frequency of occurrence) in predators taken at depths

Fig. 10. Ratio of gonad weight to total body weight of capelin from divisions 3LNO and 3M. Open and solid squares denote capelin from division 3LNO and division 3M, respectively.

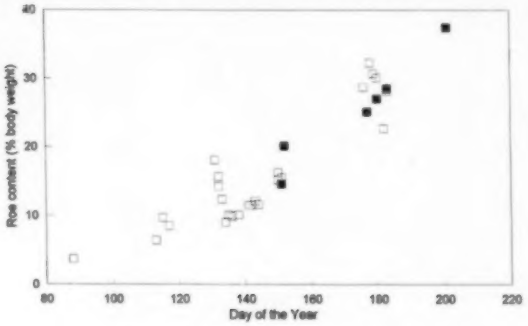
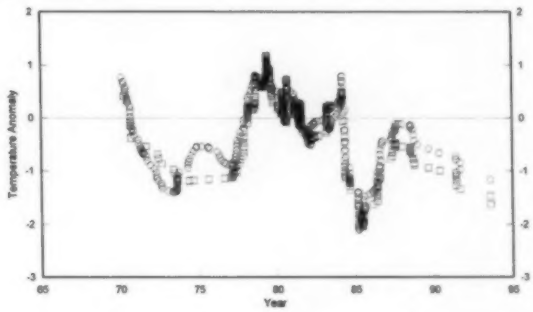


Fig. 11. Annual temperature anomalies at 100 m depth from the western (circles) and central (squares) Flemish Cap during 1970 to summer 1993.



less than 1000 m (Rodriguez-Marin et al. 1993). Although this result is consistent with the recent presence of adult capelin in the Flemish Cap region, the location of the stomach samples was reported as an aggregate of collections made in both divisions 3M and 3L.

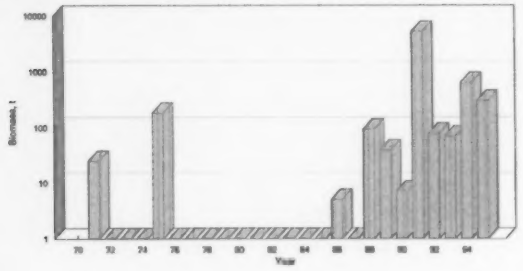
The Flemish Cap was the site of several ichthyoplankton surveys by the USSR: March–April 1959–1963, 1966, and 1970 (Serebryakov 1978) and spring and early summer 1978–1981 and 1983 (Serebryakov et al. 1987). Data from the 1959–1970 series were aggregated, but the authors reported catching 21 capelin fry during these years. At least 6 of the 41 stations occurred in and west of the Flemish Pass, with at least one station on the Grand Banks. Capelin in the area of these stations would not be an unusual occurrence. Without further details, the occurrence of capelin fry on the Flemish Cap based on the 1959–1970 surveys must be classed as doubtful. During the 1978–1983 surveys, stations were restricted to east of the Flemish Pass. One capelin larva was caught in March 1980.

Hydrographic conditions

Hydrographic sampling has been conducted on the Flemish Cap for several years and results up to summer 1993 were presented by Colbourne (1993). On the western side and central portion of the Flemish Cap, cold conditions prevailed throughout the 1970s, mid-1980s, and the late 1980s and early 1990s (Fig. 11). Negative temperature anomalies at 100 m

© 1996 NRC Canada

Fig. 12. Biomass estimates of capelin from the September RV groundfish survey of the southern Gulf of St. Lawrence (NAFO division 4T) from 1971 to 1995.



depth persisted from 1971 to 1977, and between 1978 and 1984 temperature anomalies were variable but generally positive. Intense negative anomalies began in 1985, moderated briefly in 1987, and then persisted up until the summer of 1993 (Fig. 11). The temperature patterns for this area on the Flemish Cap were similar to the patterns exhibited over the continental shelf at the same depth ranges. Over the whole area of the Flemish Cap, water temperatures, especially in the top 100 m, decreased from 1988 to 1993, and were nearly 2°C below normal. These anomalies coincided with the severe meteorological and ice conditions in the Northwest Atlantic (Colbourne 1993) and the excursion of adult capelin onto Flemish Cap. This sequence of events is reminiscent of the Scotian Shelf situation in that the occurrence of capelin outside of their normal geographic range was associated with lower than normal water temperature conditions.

Discussion

The data we have presented indicate that the occurrence of capelin on either the eastern Scotian Shelf or Flemish Cap is uncommon. The recent upsurge of capelin in these areas began in the late 1980s for the Scotian Shelf and early 1990s for the Flemish Cap and has persisted to the present. These unusual distribution patterns have occurred at a time of extreme oceanographic and meteorological conditions over the eastern Scotian Shelf, Gulf of St. Lawrence, Grand Banks, and Labrador Shelf (Drinkwater et al. 1995). Furthermore, capelin have occurred in these areas not only in the recent past (e.g., 1973 on Flemish Cap and early 1960s for the Scotian Shelf – Bay of Fundy) but also during other cold periods. On the other hand, capelin have not been recorded in these areas during times of average or above-average temperatures. There has been at least one clearly identified cold period, the mid-1980s, when the appearance of capelin in either of these two areas was minor. During this time period, capelin in survey tows on the Scotian Shelf appeared in low numbers (Fig. 5) and did not appear in any data records from Flemish Cap. On the basis of the available evidence, we conclude that capelin are generally rare in both areas, do not occur there during periods of average or above-average temperatures, and occur only during periods of below-normal temperatures. Although the latter situation appears to be a requisite condition it does not guarantee the occurrence of capelin in either of the two areas. Anomalous temperatures may simply be reflecting changes in circulation patterns as has been suggested for cooling periods on the Flemish Cap (Templeman 1976). The occurrence of capelin in these areas during anomalous temperature conditions may also depend on more than temperature and may be, for example, an interaction between temperature and population abundance.

The time series of data presented here for the Scotian Shelf cannot discriminate between a resurgence of local capelin populations or immigration. Tibbo and Humphreys (1966) interpreted the mid-1960s occurrence of capelin in the Bay of Fundy to be due to transport or migration of capelin into these areas (at an undetermined life stage) and the eventual establishment of a reproducing population. A similar set of circumstances appears to have taken place in recent times on the eastern Scotian Shelf.

The geographic extent of the recent increase in capelin abundance is not just limited to the eastern Scotian Shelf and Flemish Cap. Examination of the RV survey conducted annually since 1971 in the southern Gulf of St. Lawrence (division 4T) revealed a pattern of abundance very similar to that of the eastern Scotian Shelf (Fig. 12). There were minor peaks in abundance in the early 1970s, followed by a period of prolonged absence up to the late 1980s, and since the late 1980s, capelin have been consistently captured in the southern Gulf RV survey. Given this pattern, it is not surprising that a strong positive correlation was found between the capelin abundance time series from divisions 4VW and 4T ($r = 0.73$, $n = 25$, $p < 0.001$). As noted previously, the Gulf of St. Lawrence region has also experienced abnormally low water temperature conditions, particularly since 1990 in division 4T (Drinkwater et al. 1995).

The presence of capelin on the Flemish Cap appears to be due to immigration from the northeastern Newfoundland Shelf – Grand Banks area. The branch of the Labrador Current that splits to flow down the west side of the Flemish Cap and across the northern part of the Cap would afford a mechanism to transport larvae from the northern Grand Banks. Capelin larvae leave the Newfoundland beaches and nearshore areas within a few weeks or months of hatching and by late fall and early winter can be found widely distributed across the northern Grand Banks. Thus, biological and physical opportunities exist for larval dispersal from the Grand Banks onto the Flemish Cap. It is also possible that juvenile and adult capelin are influenced in a similar manner. On the basis of our data, this phenomenon would occur only during cold periods, suggesting an increasing influence of the Labrador Current to the east. Recall that the average temperature conditions on the Flemish Cap are suitable for capelin (see earlier section on Flemish Cap hydrography), supporting our suggestion that a limiting factor for the occurrence of capelin in this region is a means of transport from the Grand Banks.

The length-frequency data from both the Scotian Shelf and Flemish Cap indicate that the fish were older, mature fish. On the Scotian Shelf, polymodality in the size-frequency distributions (Table 1) is a strong indicator of local recruitment (spawning) in the area. On the Flemish Cap, the presence of very ripe fish in late June and early July 1993 (Fig. 10) suggests that spawning likely occurred. It is possible, however, that maturing fish detected earlier would have migrated west to the inshore spawning areas or to the spawning area on the southern Grand Banks (Carscadden et al. 1989). The presence of these capelin on the Flemish Cap could explain, in part, the

© 1996 NRC Canada

Fig. 13. Mean numbers per tow of Greenland halibut, checker eelpout, and capelin for division 4VW during RV summer surveys on the Scotian Shelf. Also shown are median bottom temperatures east of 61°W longitude. All data were smoothed using a 3-year, centre-weighted running mean to highlight the low-frequency trends in the data.

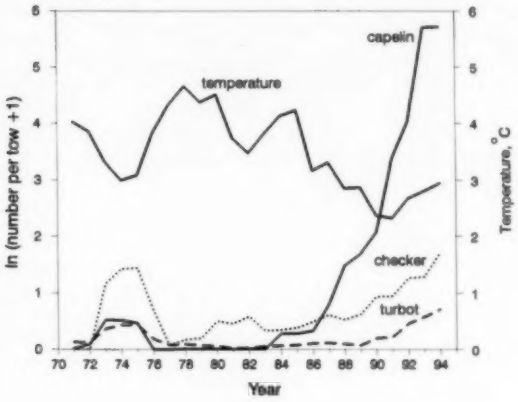
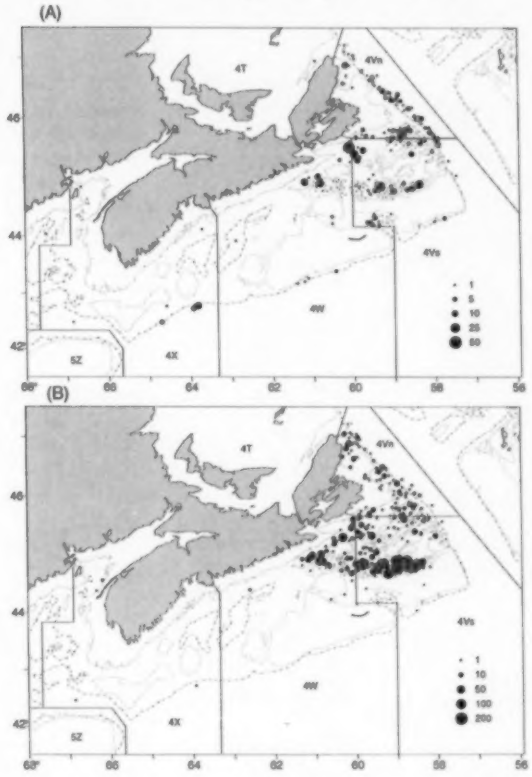


Fig. 14. Geographic distribution of Greenland halibut (A) and checker eelpout (B) depicted as expanding symbols of catch per tow from the summer RV surveys for all years combined.



dramatic decline in estimates of abundance of immature and maturing capelin from May acoustic surveys observed beginning in 1991, declines that were not witnessed in the inshore spawning areas. In a discussion of this observation, Shackell et al. (1994) suggested that a delay in maturation of capelin, mediated by low ocean temperatures, delayed the migration of capelin into the acoustic survey area. This delay of the migration into the survey area was compounded by the fact that the annual survey was fixed in time. It was suggested that some capelin were either in and around the ice and inaccessible to acoustic survey gear (a suggestion that was supported by anecdotal evidence) or elsewhere outside the survey area. The occurrence of capelin on the Flemish Cap, which is well to the east of the historical survey area (easterly survey boundary about 49°W), at the same time as the acoustic survey, is consistent with the above explanation. Our observations on the maturation schedule of capelin on the Flemish Cap (Fig. 10) did not indicate delayed maturation. However, this observed normal maturation rate and late spawning inshore during 1993 are not inconsistent because of the greater migration distance that capelin would have faced from the Flemish Cap. Further, it is tempting to speculate that some of the decline in capelin abundance measured during the offshore acoustic surveys may be due to an extensive migration into the Gulf of St. Lawrence, possibly even extending into division 4T. The abrupt increase in capelin abundance from 6 t in 1990 to over 5000 t in 1991 witnessed in the division 4T RV survey time series (Fig. 12) is consistent with the possibility that a large-scale migration of capelin had occurred.

As we have shown, capelin seem to be able to redistribute themselves relatively rapidly over an extensive geographical area in response to changing environmental conditions. During the same time period, the distributions of some groundfish species in the Newfoundland Shelf – Grand Banks area have also changed, although we know of no evidence to indicate that this shift has been as extensive (e.g., as far as the Flemish Cap) as that of capelin. For the Scotian Shelf area, we examined the results from the RV surveys and noted that few Arctic–boreal species had responded similarly to the recent, and past, cold-water conditions in that region. Out of several species considered (see Simon and Comeau 1994 for a complete description), two showed a temporal pattern of abundance similar to capelin: Greenland halibut and checker eelpout, *Lycodes vahlii*. Peak catch rates of Greenland halibut (or turbot) and checker eelpout were observed during the mid-1970s and during the 1990s, both of which correspond to periods of below-normal water temperature on the eastern Scotian Shelf (Fig. 13). The correlations between median bottom temperature and catch rate ($n = 26$) for turbot and checker eelpout were −0.35 ($p < 0.10$) and −0.50 ($p < 0.01$), respectively. If the temperature and catch-rate series are smoothed (using a 3-year running mean as depicted in Fig. 13) to enhance the low-frequency variability, the correlations increase substantially to −0.54, −0.71, and −0.66 for turbot, checker eelpout, and capelin, respectively. The distributional pattern of turbot and checker eelpout is confined to the eastern Scotian Shelf, closely resembling the patterns for capelin (Fig. 14). It should also be noted that the first occurrence of Greenland halibut in the Bay of Fundy was recorded in 1968 by Barrett (1968), and Boyar (1964) reported its occurrence off Boothbay Harbour in 1963, both records being associated with times of below-normal water temperatures in the area of capture and with the

© 1996 NRC Canada

major cooling event of the Scotian Shelf and the Gulf of Maine during the mid-1960s (Fig. 3).

The limited number of species whose geographic distribution changes extensively on ecological time scales may ultimately be linked to the physiological capacity of the species. As Colton (1972) noted during the 1952–1967 cooling event of the continental shelf waters from Nova Scotia to Long Island, the distributional patterns of several groundfish species did not change extensively, and it was suggested that spawning area and bottom type were the principal habitat requirements for species such as yellowtail flounder (*Pleuronectes ferruginneus*) and haddock (*Melanogrammus aeglefinus*). Seasonal production of blood antifreeze proteins among certain species (cod, winter flounder (*Pleuronectes americanus*), eelpout) may allow individuals to remain in nearshore waters during winter where water temperatures drop below 0°C such as in the southern Gulf of St. Lawrence and coastal Newfoundland (Goddard et al. 1992).

Capelin are a major diet item of cod in several geographic areas (e.g,. Newfoundland: Lilly 1991; Iceland: Magnusson and Palsson 1989; Norway: Hamre 1991). One would expect, therefore, that the resident cod stocks in the two target areas (as well as the southern Gulf of St. Lawrence) would benefit from the influx of new and abundant prey. The available evidence suggests that this has not occurred. On the Scotian Shelf, cod abundance has declined dramatically and weight at age (an index of condition) has shown a steady decline since the mid-1980s (Frank et al. 1994). This situation may be attributed, in part, to excessive exploitation, but we cannot rule out concurrent effects of changes in the physical environment. On the Flemish Cap, the evidence is more direct. Feeding studies between 1982 and 1992 indicated that cod were not feeding on capelin (Casas and Paz 1994). Sinclair (1994) found significant negative trends in the weight at age 7 for cod during 1978–1992 in four of eight Canadian stocks examined: cod from divisions 2K3KL, 3Pn4RS, 4T, and 4VsW. Furthermore, in 1992 weights at age 7 were between 52 and 70% of the weights observed in 1978 (Sinclair 1994).

How long will capelin persist on the Scotian Shelf and Flemish Cap? Their presence is clearly linked to the cooling trends in both areas, and we would expect capelin to continue in these areas as long as water temperatures remain below normal. Given the recent warming of some of the deep basins in and around the Scotian Shelf (Cabot Strait and Emerald Basin) owing to the presumed changes in the slope water characteristics (Drinkwater et al. 1995), it is entirely possible that the capelin population will shortly disappear. However, as shown by the 1995 survey results, a continuing presence of capelin on the eastern Scotian Shelf is readily apparent. Capelin were once again reported as by-catch in the 1994 Flemish Cap shrimp fishery.

Finally, was our original suggestion that capelin may serve as an instructive analog for interpreting recent events in the Newfoundland fishery (i.e., the collapse of northern cod) correct? We believe the answer is yes and that it is entirely possible that redistribution of northern cod to areas outside of the current management unit (division 2J3KL) could have contributed to the rapid decline in cod abundance that was reported in the early 1990s. The geographic scale of such a redistribution could be as large as we speculate here for capelin or even greater if the observations made by Earll (1880) are indeed correct.

Acknowledgements

We thank the following individuals who supplied biological data or helped in the preparation of the manuscipt: J. Firth, D. Kulka, G. Lilly, and M. Hynes, DFO, St. John's, Newfoundland; Antonio Vasques, Instituo de Investigaciones Marinas, Muelle de Bouzas, 36208 Vigo, Spain; and G. Chouinard, DFO, Moncton, N.B. We are also grateful to K. Drinkwater, B. Petrie, and E. Colbourne for supplying hydrographic data and associated data products for the Scotian Shelf and Flemish Cap.

References

Akenhead, S.A., Carscadden, J.E., Lear, H., Lilly, G.R., and Wells, R. 1982. Cod–capelin interactions off northeast Newfoundland and Labrador. *In* Multispecies approaches to fisheries management advice. *Edited by* M.C. Mercer. Can. Spec. Publ. Fish. Aquat. Sci. No. 59. pp. 141–148.

Albikovskaya, L.K., and Gerasimova, O.V. 1989. Feeding and food interrelations between cod (*Gadus morhua*) and beaked redfish (*Sebastes mentella* T.) on Flemish Cap. NAFO SCR Doc. No. 89/9. Ser. No. N1573. Northwest Atlantic Fisheries Organization, Dartmouth, N.S.

Anonymous. 1995. Report of the Cod and Climate BACKWARD-FACING Workshop. ICES CM 1995/A:7. Ref. C,G,H,J,L. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Barrett, B.E. 1968. First occurrence of Greenland halibut, *Reinhardtius hippoglossoides*, in the Bay of Fundy. J. Fish. Res. Board Can. **25**: 2721–2722.

Bigelow, H.B., and Schroeder, W.C. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildl. Serv. Bull. No. 53. (Fish. Bull. No. 74.)

Black, G.A. 1991. ACON: documentation for ACON version 7.27. Department of Fisheries and Oceans, Dartmouth, N.S.

Borokov, V.A., Bulatova, A.Y., Chumakov, A.K., Savvatimsky, P.I., and Tevs, I.I. 1989. Bottom water effects on the distribution and density of bottom fish in NAFO Subarea 3. NAFO SCR Doc. No. 89/87. Ser. No. N1671. North Atlantic Fisheries Organization, Dartmouth, N.S.

Boyar, H.C. 1964. Occurrence of Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), in shallow waters in the Gulf of Maine. Copeia, 1964: 232–233.

Carscadden, J.E., and Miller, D.S. 1981. Analytical assessment of the capelin stock in Subarea 2 + Div. 3K using SCAM. NAFO SCR Doc. No. 81/4. Ser. No. N268. North Atlantic Fisheries Organization, Dartmouth, N.S.

Carscadden, J.E., Frank, K.T., and Miller, D.S. 1989. Capelin (*Mallotus villosus*) spawning on the Southeast Shoal: influence of physical factors past and present. Can. J. Fish. Aquat. Sci. **46**: 1743–1754.

Carscadden, J.E., Nakashima, B.S., and Frank, K.T. 1996. Interannual variation in the timing of spawning in northwest Atlantic capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. In press.

Casas, J.M., and Paz, J. 1994. Diet of Flemish Cap cod with particular reference to predation on redfish: 1988–93. NAFO SCR Doc. No. 94/24. Ser. No. 2390. North Atlantic Fisheries Organization, Dartmouth, N.S.

Colbourne, E. 1993. Oceanographic conditions on the Flemish Cap during the summer 1993, with comparisons to the long-term average. NAFO SCR Doc. No. 93/107. Ser. No. N2300. North Atlantic Fisheries Organization, Dartmouth, N.S.

Colton, J.B., Jr. 1972. Temperature trends and the distribution of

© 1996 NRC Canada

groundfish in continental shelf waters, Nova Scotia to Long Island. Fish. Bull. **70**: 637–657. North Atlantic Fisheries Organization, Dartmouth, N.S.

Cushing, D.H. 1982. Climate and fisheries. Academic Press, London.

deYoung, B., and Rose, G.A. 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can. J. Fish. Aquat. Sci. **50**: 2729–2741.

Drinkwater, K.F., and Trites, R.W. 1986. Monthly means of temperature and salinity in the Grand Banks region. Can. Tech. Rep. Fish. Aquat. Sci. No. 1450.

Drinkwater, K.F., Colbourne, E., and Gilbert, D. 1995. Overview of environmental conditions in the northwest Atlantic in 1994. NAFO SCR Doc. No. 95/43. North Atlantic Fisheries Organization, Dartmouth, N.S.

Dunbar, M.J. 1966. The sea waters surrounding the Québec–Labrador Peninsula. Cah. Geogr. Que. **10**(9): 13–36.

Earll, R.E. 1880. A report on the history and present condition of the shore cod-fisheries of Cape Ann, Mass., together with notes on the natural history and artificial propagation of the species. *In* Report of the U.S. Fisheries Commission for 1878. Vol. 6. Government Printing Office, Washington, D.C. pp. 685–740.

Frank, K.T., Drinkwater, K.F., and Page, F.H. 1994. Possible causes of recent trends and fluctuations in Scotian Shelf/Gulf of Maine cod stocks. ICES Mar. Sci. Symp. **198**: 110–120.

Goddard, S.V., Kao, M.H., and Fletcher, G.L. 1992. Antifreeze production, freeze resistance, and overwintering of juvenile northern Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **49**: 516–522.

Halliday, R.G., and Koeller, P.A. 1981. A history of Canadian groundfish trawling surveys and data usage in ICNAF Division 4TVWX. *In* Bottom Trawl Surveys: Proceedings of a Workshop, Ottawa, Ont, 12–14 November 1980. *Edited by* W.G. Doubleday and D. Rivard. Can. Spec. Publ. Fish. Aquat. Sci. No. 58. pp. 27–41.

Hamre, J. 1991. Interrelation between environmental changes and fluctuating fish populations in the Barents Sea. *In* Long-term variability of pelagic fish populations and their environment. *Edited by* T. Kawasaki, S. Tanaka, Y. Toba, and D. Taniguchi. pp. 259–270.

Huntsman, A.G. 1922. The fishes of the Bay of Fundy. Contrib. Can. Biol. 1921(3): 49–72.

Hutchings, J.A., and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. **51**: 2126–2146.

Jangaard, P.M. 1974. The capelin (*Mallotus villosus*): biology, distribution, exploitation, utilization, and composition. Bull. Fish. Res. Board Can. No. 186.

Konstantinov, K.G., Turuk, T.N., and Plekhamova, N.A. 1985. Food links of some fishes and invertebrates on Flemish Cap. NAFO Sci. Coun. Stud. **8**: 39–48.

Lilly, G.R. 1987. Synopsis of research related to recruitment of Atlantic cod (*Gadus morhua*) and Atlantic redfishes (*Sebastes* sp.) on Flemish Cap. NAFO Sci. Coun. Stud. **11**: 109–122.

Lilly, G.R. 1991. Interannual variability in predation by cod (*Gadus morhua*) on capelin (*Mallotus villosus*) and other prey off southern Labrador and northeastern Newfoundland. ICES Mar. Sci. Symp. **193**: 133–146.

Lilly, G.R., and Davis, D.J. 1993. Changes in the distribution of capelin in Divisions 2J, 3K and 3L in the autumns of recent years, as inferred from bottom-trawl by-catches and cod stomachs examinations. NAFO SCR Doc. No. 93/54. Ser. No. 2237. North Atlantic Fisheries Organization, Dartmouth, N.S.

Magnusson, K.G., and Palsson, O.K. 1989. Trophic ecological relationships of Icelandic cod. Rapp. P-V. Reun. Cons. Int. Explor. Mer, **188**: 206–224.

Miller, D.S. 1992. Results of an acoustic survey for capelin (*Mallotus villosus*) in NAFO division 3L in 1992. NAFO SCR Doc. No. 92/57. Ser. No. N2110. North Atlantic Fisheries Organization, Dartmouth, N.S.

Miller, D.S. 1993. Observations and studies on SA2 + Div. 3K capelin in 1992. CAFSAC Res. Doc. No. 93/10. Canadian Atlantic Fisheries Scientific Advisory Committee, Dartmouth, N.S.

Miller, D.S. 1994. Results from an acoustic survey for capelin (*Mallotus villosus*) in NAFO Divisions 2J3KL. *In* Capelin in SA2 + Div. 3KL. *Edited by* J.E. Carscadden. DFO Atl. Fish. Res. Doc. No. 94/18. pp. 91–98. Department of Fisheries and Oceans, Dartmouth, N.S.

Nakashima, B.S. 1994. The inshore capelin fishery in NAFO Div. 3KL in 1993. *In* Capelin in SA2 + Div. 3KL. *Edited by* J.E. Carscadden. DFO Atl. Fish. Res. Doc. No. 94/18. pp. 6–34. Department of Fisheries and Oceans, Dartmouth, N.S.

Noskov, A.S., Zakharov, G.P., and Sidorenko, I.N. 1963. USSR research report 1962. *In* ICNAF Redbook 1963. Part II. International Commission for the Northwest Atlantic Fisheries, Dartmouth, N.S. pp. 79–101.

Page, F.H., and Losier, R.J. 1994. Temperature variability during Canadian bottom trawl summer surveys conducted in NAFO Division 4VWX, 1970–1992. ICES Mar. Sci. Symp. **198**: 323–331.

Paz, P.J., Alvarez, F.J.V., Arroyo, A.F., Sanchez, J.M.C., and deCardenes, E. 1989. The feeding of American plaice (*Hippoglossoides platessoides*), redfish (*Sebastes marinus*) and cod (*Gadus morhua*) in the Flemish Cap during July 1988. NAFO SCR Doc. No. 89/45. Ser. No. N1622. North Atlantic Fisheries Organization, Dartmouth, N.S.

Paz, J., Casas, M., and Perez-Gandaras, G. 1991. The feeding of cod (*Gadus morhua*) on Flemish Cap, 1989–90. NAFO SCR Doc. No. 91/31. Ser. No. N1911. North Atlantic Fisheries Organization, Dartmouth, N.S.

Petrie, B., and Drinkwater, K. 1993. Temperature and salinity variability on the Scotian Shelf and in the Gulf of Maine 1945–1990. J. Geophys. Res. **98**: 20 079 – 20 089.

Pitt, T.K. 1958. Distribution, spawning and racial studies of the capelin, *Mallotus villosus* (Muller), in the offshore Newfoundland areas. J. Fish. Res. Board Can. **15**: 275–293.

Rodriguez-Marin, E., Punzón, A., and Paz, J. 1993. Greenland halibut (*Reinhardtius hippoglossoides*) feeding in Flemish Pass: NAFO Divisions 3LM. NAFO SCR Doc. No. 93/18. Ser. No. N2195. North Atlantic Fisheries Organization, Dartmouth, N.S.

Serebryakov, U.P. 1978. Ichthyoplankton from the Flemish Cap Bank. ICNAF Res. Doc. No. 78/VI/18. Ser. No. 5172. International Commission for the Northwest Atlantic Fisheries, Dartmouth, N.S.

Serebryakov, U.P., Astafjeva, A.V., Aldonov, V.K., and Chumako, A.K. 1987. USSR Ichthyoplankton investigations on Flemish Cap, 1978–83. NAFO Sci. Coun. Stud. **11**: 7–21.

Shackell, N.L., Carscadden, J.E., and Miller, D.S. 1994. Migration of pre-spawning capelin (*Mallotus villosus*) as related to temperature on the northern Grand Bank, Newfoundland. ICES J. Mar. Sci. **51**: 107–114.

Shelton, P.A., Carscadden, J.E., and Hoenig, J.M. 1993. Risk evaluation of the 10% harvest rate procedure for capelin in NAFO Div. 3L. *In* Risk evaluation and biological reference points for fisheries management. *Edited by* S.J. Smith, J.J. Hunt, and D. Rivard. Can. Spec. Publ. Fish. Aquat. Sci. No. 120. pp. 193–201.

Simon, J.E., and Comeau, P.A. 1994. Summer distribution and abundance trends of species caught on the Scotian Shelf from 1970–92, by the research vessel groundfish survey. Can. Tech. Rep. Fish. Aquat. Sci. No. 1953.

Sinclair, A. 1994. Recent declines in cod stocks in the northwest Atlantic. NAFO SCR Doc. No. 94/73. North Atlantic Fisheries Organization, Dartmouth, N.S.

Smith, S.J., and Somerton, G.D. 1981. STRAP: a user-oriented computer analysis system for groundfish research trawl survey data. Can. Tech. Rep. Fish. Aquat. Sci. No. 1030.

© 1996 NRC Canada

Soutar, A., and Isaacs, J.D. 1974. Abundance of pelagic fish during the 19th and 20th centuries as recorded in anaerobic sediments off California. Fish. Bull. **72**: 257–275.

Templeman, W. 1976. Biological and oceanographic background of Flemish Cap as an area for research on the reasons for year-class success and failure in cod and redfish. ICNAF Res. Bull. No. 12. International Commission for the Northwest Atlantic Fisheries, Dartmouth, N.S. pp. 91–117.

Tibbo, S.N., and Humphreys, R.D. 1966. An occurrence of capelin (*Mallotus villosus*) in the Bay of Fundy. J. Fish. Res. Board Can. **23**: 463–467.

Turuk, T.N. 1981. Year-to-year and seasonal fluctuations in feeding biological indices of the Flemish Cap Bank cod. NAFO SCR Doc. No. 81/IV/76. Ser. No. N361. North Atlantic Fisheries Organization, Dartmouth, N.S.

Turuk, T.N., and Postolaky, A.I. 1980. Feeding and food relations of some fish species in the Labrador and Newfoundland areas. NAFO SCR Doc. No. 80/VI/70. Ser. No. N118. North Atlantic Fisheries Organization, Dartmouth, N.S.

Vazquez, A. 1993. Results from bottom trawl survey of Flemish Cap in July 1992. NAFO SCR Doc. No. 93/19. Ser. No. 2196. North Atlantic Fisheries Organization, Dartmouth, N.S.

Winters, G.H. 1982. Life history and geographical patterns of growth in capelin, *Mallotus villosus*, of the Labrador and Newfoundland areas. J. Northwest Atl. Fish. Sci. **3**: 105–114.

© 1996 NRC Canada

Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids

D.K. Hawkins and T.P. Quinn

Abstract: Critical swimming velocities were determined for two hatchery stocks of cutthroat trout (*Oncorhynchus clarki clarki*), one hatchery stock of steelhead trout (*Oncorhynchus mykiss*), and reciprocal cutthroat–steelhead hybrids from both cutthroat stocks. Steelhead had the highest critical swimming velocity (7.69 body lengths/s), cutthroat had the lowest (5.58 and 6.69 body lengths/s for the two stocks), and hybrids were intermediate (7.14 and 7.22 body lengths/s). Consistent with the significant differences in swimming performance, principal components analysis revealed that cutthroat had shorter and narrower caudal regions than steelhead and that hybrids were morphologically intermediate. The morphology of hatchery populations compared with that of wild juvenile cutthroat and steelhead allowed us to determine if the interspecific morphological differences observed in hatchery-reared fish were representative of the species and did not result from hatchery practices, and to assess the validity of extrapolating the swimming performance results to wild fish. Although wild fish were generally more fusiform than hatchery fish of both species, the relative differences between cutthroat and steelhead shapes were the same for both hatchery-reared and wild fish. We conclude that hybrids are intermediate to the parental species in both swimming performance and morphology and that the potential for a competitive advantage exists.

Résumé : Nous avons calculé les vitesses critiques de nage pour deux stocks d'élevage de truite fardée (*Oncorhynchus clarki clarki*), un stock d'élevage de truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) et des hybrides réciproques de ces deux truites provenant des deux stocks de truite fardée. La truite arc-en-ciel anadrome présentait la vitesse critique de nage la plus élevée (7,69 longueurs corporelles/s), la truite fardée la plus basse (5,58 et 6,69 longueurs corporelles/s pour les deux stocks), tandis que les hybrides donnaient des résultats intermédiaires (7,14 et 7,22 longueurs corporelles/s). Conformément aux différences significatives dans la performance de nage, l'analyse des composantes principales a révélé que la truite fardée présentait une région caudale plus courte et plus étroite que la truite arc-en-ciel anadrome, et que les hybrides possédaient des caractéristiques morphologiques intermédiaires. La comparaison de la morphologie des populations d'élevage à celle de juvéniles sauvages de truite fardée et de truite arc-en-ciel anadrome nous a permis de déterminer si les différences morphologiques interspécifiques observées chez les poissons d'élevage étaient représentatives de l'espèce et non pas dues aux pratiques piscicoles, et d'évaluer la validité d'une extrapolation aux poissons sauvages des résultats sur la performance de nage. Si les poissons sauvages étaient en général plus fusiformes que les spécimens d'élevage chez les deux espèces, les différences relatives entre la forme des deux espèces étaient les mêmes chez les poissons d'élevage et les poissons sauvages. Nous en concluons que les hybrides ont une position intermédiaire entre les espèces parentes tant pour la performance de nage que pour la morphologie, et qu'un avantage compétitif peut ainsi exister.
[Traduit par la Rédaction]

Introduction

Coastal cutthroat (*Oncorhynchus clarki clarki*) and steelhead trout (*Oncorhynchus mykiss*) coexist throughout much of their native ranges. Although much is yet to be learned about the timing of phyletic divergence (Utter and Allendorf 1994), Behnke (1992) concluded that cutthroat and steelhead shared a common ancestor as recently as 2 million years ago. As would be expected from this relatively recent divergence, cutthroat and steelhead do not have many unique derived characteristics. However, differences at protein loci (Campton and Utter 1985; Leary et al. 1987), in mitochondrial DNA sequence (Wilson et al. 1985), in karyotype (Gold 1977), and in morphology (Hartman 1956; Stearley and Smith 1993) can be used to distinguish between the species.

Prior to 1981, hybridization between these two species was thought to be uncommon and had not been formally documented (Utter 1981; Verspoor 1988). However, Campton (1981) and Campton and Utter (1985) found a significant number of hybrids in two streams in the Puget Sound area of the state of Washington, indicating that hybridization occurs between coastal cutthroat and steelhead. Hybridization is cause for some concern because it may contribute to the decline of western trout species (Busack and Gall 1981), and many cutthroat populations currently are declining (Johnston and Mercer 1976; Trotter 1987; Nehlsen et al. 1991; Johnson et al. 1994).

Received May 24, 1995. Accepted February 15, 1996.
J12928

D.K. Hawkins[1] and T.P. Quinn. School of Fisheries, University of Washington, P.O. Box 357980, Seattle, WA 98195, U.S.A.

[1] Author to whom all correspondence should be addressed.
e-mail: dkh@fish.washington.edu

© 1996 NRC Canada

There are several reproductive isolating mechanisms, both pre- and post-mating, which maintain the species differences. Spawning adults are generally separated both temporally and spatially (Behnke 1972; Trotter 1987; Burgner et al. 1992). In western Washington, steelhead often spawn later in the season, using the deeper, faster water of larger rivers, whereas cutthroat spawn earlier in smaller headwaters (Johnston and Mercer 1976). Steelhead are also often much larger, which could lead to assortative mating or possibly to directionality in pair formation in the event of heterospecific matings (Foote and Larkin 1988). Furthermore, cutthroat and steelhead have different numbers of chromosomes (68 and 58–60, respectively; Behnke 1970), which could lead to mispairing of chromosomes and decreased viability in hybrid progeny.

Hybridization may be thought of as the breakdown of isolating mechanisms between two reproductively isolated species or populations (Mayr 1963). In fishes, hybridization is common within many families, owing in large part to the frequency of external fertilization and weak pair bonds (Hubbs 1955). There are many examples of hybridization among salmonids (Chevassus 1979; Paiute and Lahontan cutthroat, Busack and Gall 1981; golden and rainbow trout, Halliburton et al. 1983; arctic char and brook trout, Hammar et al. 1991; Apache, Gila, cutthroat, and rainbow trout, Dowling and Childs 1992; Atlantic salmon and brown trout, Jordan and Verspoor 1993). However, relative performance of the hybrid and parental offspring has seldom been compared in salmonids. In a review of the fitness of hybrids relative to their parents, Arnold and Hodges (1995) provided examples from many genera and concluded that hybrids are not uniformly less fit than the parental species. The relative fitness of the F_1 progeny determines whether occasional hybridization strengthens the isolating mechanisms or leads to introgression of the gene pools (Mayr 1963).

Sustained swimming ability is an important component of fitness because it affects a fish's habitat utilization, ability to perform extended migrations, and vulnerability to predation (Bams 1967; Tsuyuki and Williscroft 1977; Taylor and McPhail 1985; Bisson et al. 1988). Furthermore, swimming ability is correlated with body shape (Thomas and Donahoo 1977; Taylor and McPhail 1985). Taylor and Foote (1991) related differences in swimming ability among juvenile sockeye, kokanee, and their hybrids to differences in morphology and differences in the extent of adult migrations. Sockeye salmon, the anadromous form of *Oncorhynchus nerka*, undertake extensive migrations whereas kokanee do not. The sockeye body shape is consistent with the need for superior sustained swimming performance (longer, narrower body and longer caudal region). Taylor and McPhail (1985) showed similar differences between juvenile coastal and interior forms of coho salmon (*Oncorhynchus kisutch*). Interior coho, which undertake more extensive migrations as adults, are more fusiform and are better swimmers. Juvenile cutthroat and steelhead utilize different stream habitats; steelhead are generally found in waters with higher velocities (Thomas and Donahoo 1977; Bisson et al. 1988). There are also differences in the duration and extent of the adult marine migrations. Cutthroat generally spend only the summer in the ocean and are thought to remain near shore, whereas steelhead are at sea for 1 or more years and migrate long distances (Giger 1972; Trotter 1989; Burgner et al. 1992; Johnson et al. 1994). Consistent with these differences in juvenile habitat and adult migrations, steelhead are more cylindrical with short median fins and large paired fins useful for holding position in fast water (Hartman 1956; Bisson et al. 1988). Because of these differences in body shape, juvenile habitat preferences, and adult migrations between cutthroat and steelhead, we hypothesized that steelhead would be better swimmers than cutthroat.

The relative swimming performance of cutthroat, steelhead, and their hybrids is unknown. Reduced swimming ability could put hybrids at a competitive disadvantage compared with both cutthroat and steelhead, resulting in a barrier to gene flow. Enhanced swimming ability, on the other hand, could result in a competitive advantage for the hybrids, increasing the potential for introgression. The consequences of intermediate swimming ability of hybrids, relative to the parental species, would depend on the life-history pattern of the hybrids. Hybrids that adopt steelhead freshwater habitat and marine migrations could be competitively inferior because of selection for enhanced swimming ability in steelhead. However, hybrids that adopt cutthroat behaviors could be competitively superior to cutthroat. Bisson et al. (1988) described cutthroat as lacking morphological adaptations to either fast or slow water and suggested that this could be in part responsible for their displacement by other salmonids. If hybrids are able to attain higher critical swimming velocities than cutthroat, perhaps they could also displace cutthroat from more desirable habitat.

In this study, critical swimming velocities were determined for cutthroat, steelhead, and their hybrids to assess relative swimming abilities. Morphometric variation was also analyzed to determine if differences in swimming ability were related to differences in body shape. Hybrid performance and morphology were compared with the parental species to determine if cutthroat–steelhead hybrids were intermediate in both swimming ability and body shape. Finally, the variations in body morphology of the cutthroat and steelhead hatchery populations used in the swimming tests were compared with the body morphology of juvenile cutthroat and steelhead collected in the wild, as environmentally induced morphological differences between hatchery and wild fish could affect the ability to relate swimming performance differences determined with hatchery fish to wild populations (Taylor 1986; Swain et al. 1991).

Materials and methods

Adults from two coastal cutthroat trout stocks and one steelhead trout stock were used to create the progeny for this experiment. One stock of cutthroat and the steelhead were obtained from the Washington State Hatchery at Aberdeen, Washington. The other cutthroat stock came from the Washington State Hatchery at Shelton, Washington. The Lake Aberdeen cutthroat were identified as having some past genetic influence by subsequent protein electrophoresis, but seven of the eight adults used for gamete collection had only cutthroat trout alleles at all loci examined. Both stocks of cutthroat originated from anadromous fish, but neither stock is currently released. Brood stocks are maintained at the hatcheries and spawned as 3 year olds. The steelhead are released, and the hatchery stock is maintained using returning adults. Therefore, the steelhead undergo some selection for successful migration, whereas the cutthroat do not. Eggs and milt were obtained on 18 January (Aberdeen cutthroat and steelhead) and 27 January (Shelton cutthroat and Aberdeen steelhead), 1994, from

© 1996 NRC Canada

Fig. 1. Genetic design for cutthroat, steelhead, and hybrid progeny tested in swimming tunnels. In all fish types the dam is listed first and subscripts indicate origin of parent. C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton.

Aberdeen Hatchery (repeated four times, total 16 families)

	C_aC_a cutthroat dam	SS steelhead dam
C_aC_a cutthroat sire	C_aC_a pure cutthroat	S_aC_a paternal cutthroat hybrid
SS steelhead sire	C_aS_a maternal cutthroat hybrid	ESS early pure steelhead

Shelton Hatchery (repeated 6 times, total 24 families)

	C_sC_s cutthroat dam	SS steelhead dam
C_sC_s cutthroat sire	C_sC_s pure cutthroat	S_sC_s paternal cutthroat hybrid
SS steelhead sire	C_sS_s maternal cutthroat hybrid	LSS late pure steelhead

the two hatcheries and transported to the hatchery at the University of Washington, School of Fisheries, Seattle. The eggs from each female were divided into two lots, one fertilized by a conspecific male and one fertilized by a heterospecific male. The same male was used to fertilize one conspecific and one heterospecific lot of eggs. Each pair of cutthroat and steelhead females shared a pair of cutthroat and steelhead males so that the hybrid families created by a pair of females were maternal or paternal half-sibs to the pure cutthroat and steelhead families. Ten pairs of steelhead, four pairs of Aberdeen cutthroat, and six pairs of Shelton cutthroat were used, resulting in 40 families, 10 of each major cross type. The four major cross types were CC, pure cutthroat; CS, hybrids with cutthroat dams; SC, hybrids with steelhead dams; and SS, pure steelhead. Because the Shelton cutthroat were spawned 1 week later than the Aberdeen cutthroat, the steelhead used in creating the Shelton families were also collected 1 week later. Therefore, each major cross type had two minor cross types that could be compared. The eight minor cross types were C_aC_a, Aberdeen cutthroat; C_sC_s, Shelton cutthroat; C_aS_a, hybrids with Aberdeen cutthroat dams, C_sS_s, hybrids with Shelton cutthroat dams; S_aC_a, hybrids with Aberdeen cutthroat sires; S_sC_s, hybrids with Shelton cutthroat sires; ESS, early Aberdeen steelhead used in crosses with Aberdeen cutthroat; and LSS, late Aberdeen steelhead used in crosses with Shelton cutthroat (Fig. 1).

Families were incubated separately in randomly assigned Heath trays using dechlorinated city water maintained at 10 ± 1°C. As each family reached the swim-up stage in late March, they were transferred to 19-L rearing buckets supplied with Lake Washington water, which warmed from 10 to 19°C as the season progressed. The fish were fed Moore–Clark salmon diet three times daily for 1 month and once daily thereafter to satiation. Throughout the rearing and testing periods the fish experienced controlled lighting (12 h light : 12 h dark) to prevent changes in morphology caused by exogenous day-length cues. Because of heavy mortalities in both pure Aberdeen cutthroat and pure Shelton cutthroat, additional cutthroat were obtained from the hatcheries as fry on 31 May and 13 June, respectively. These fry were a mixture of the progeny produced at the hatchery that year and therefore represented many families. At the School of Fisheries hatchery these fish were maintained in separate 19-L containers in the same water and under the same husbandry regimen as the initial crosses until testing.

Critical swimming velocities were determined in a modified Blazka-type swim chamber (Smith and Newcomb 1970; Flagg 1981; Moser et al. 1990) consisting of a Plexiglas swimming tunnel (16 cm diameter, 71 cm long) contained within another larger Plexiglas tube (24 cm diameter). Water flow was generated by an impeller at the rear of the chamber controlled by a variable-speed, 1-hp (1 hp = 745.70 W) DC motor. Another impeller at the front of the chamber and a bundle of plastic straws inserted into the chamber reduced the friction at the side of the chamber and created a more equally distributed flow (Carpenter 1987). An electrified screen placed before the rear impeller kept the fish from contacting the propeller or leaving the testing chamber. The electric current to the screen, controlled by a rheostat, prevented the fish from resting on the screen.

Fish were tested at 4–5 months of age (9 July – 29 August), in lake water at the same temperature as their rearing water. Testing occurred from 0.5 h after lights on until 0.5 h before lights off. On each day, fish from the different types were tested in a random order to minimize any effect of time of day on swimming performance. For each test a fish was randomly selected from the rearing bucket. The fork length was estimated by placing the fish in a small, water-filled plastic tube containing a ruler before it was placed in the swimming tunnel. The estimated fork length was used to calculate water velocities in body lengths per second (bl/s) to avoid exposing the fish to anesthetic or excessive handling prior to testing. Basing water velocities on fork length allowed comparison among fish of different sizes because swimming ability has been shown to be related to size (Brett 1965; Thomas and Donahoo 1977). After a 5-min acclimation period, the water velocity was gradually increased from zero to the initial velocity of 3 bl/s over a 5-min period, then maintained at this speed for 15 min. Each 15 min thereafter, the velocity was increased by 1 bl/s over 1 min. This procedure was continued until the fish was no longer able to maintain position and fell against the electrified screen. At this point, the electricity and water current were turned off, the time elapsed in the last 15-min period was recorded, and the fish was removed from the tunnel.

After the test, the fish was anaesthetized with MS–222 buffered with sodium bicarbonate, morphological measurements were taken, the adipose fin was clipped to prevent retesting, and the fish was returned to the rearing bucket. The regression relationship between the estimated fork length and the actual fork length indicated that the estimated lengths were accurate and unbiased over the size range of fish tested ($r^2 = 0.99$, $n = 133$, slope = 0.96, y intercept = 3.33 mm). Therefore, swimming velocities were not corrected for differences between the two measurements.

Critical swimming velocity (U_{crit}; body lengths per second) was calculated using the formula given by Brett (1964):

$$U_{crit} = V_p + (T_f/T_i)V_i$$

where V_p is the velocity at which the fish last swam for the full 15-min period, V_i is the velocity increment (1 bl/s), T_f is the elapsed time from the last velocity increase to fatigue, and T_i is the time between velocity increases (15 min).

We anticipated that cutthroat and steelhead reared under common conditions would display species-specific morphological differences and that these might be associated with patterns of swimming performance. However, the performance and morphology of the hybrids could resemble one or the other parental species or be different from either. Therefore, we measured the morphology of all fish tested to relate body shape and swimming performance to each other and to the parents.

© 1996 NRC Canada

Table 1. Sample sizes and mean critical swimming velocities (bl/s, mean ± SE) for groups tested in the swimming tunnel, prior to pooling between minor cross types.

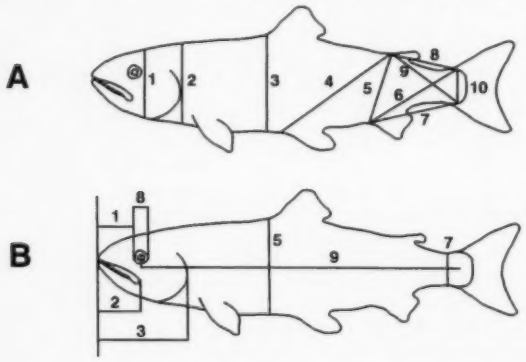
Fish type	No. tested	No. of families	Mean critical swimming velocity[a]
C_aC_a Aberdeen cutthroat received as eggs	2	2	6.38±0.96 (1)
C_aC_a Aberdeen cutthroat received as fry	11	Unknown[b]	6.74±0.23 (1)
C_sC_s Shelton cutthroat received as eggs	12	3	5.71±0.22 (2)
C_sC_s Shelton cutthroat received as fry	11	Unknown[b]	5.45±0.20 (2)
C_aS_a maternal Aberdeen cutthroat hybrid	9	3	7.29±0.27 (3)
C_sS_a maternal Shelton cutthroat hybrid	22	3	7.08±0.18 (3)
S_aC_a paternal Aberdeen cutthroat hybrid	15	3	7.40±0.24 (4)
S_aC_s paternal Shelton cutthroat hybrid	20	4	7.09±0.12 (4)
ESS early spawned Aberdeen steelhead	16	3	7.50±0.27 (5)
LSS late spawned Aberdeen steelhead	15	3	7.90±0.18 (5)
Total	133	24[b]	na

Note: In all fish types the dam is listed first and subscripts indicate origin of parent: C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton. na, not applicable.

[a]Values in parentheses indicate groups pooled on the basis of *t* tests.

[b]Cutthroat received as fry were from a large raceway containing mixed fry from the fish spawned at the hatchery that year.

Fig. 2. (A) Morphological traits used in principal components analysis of fish tested in swimming tunnels. 1, head depth 1 (HD1); 2, head depth 2 (HD2); 3, body depth at insertion of dorsal fin (PELVDF); 4, insertion of pelvic fin to insertion of adipose fin (PELVAD); 5, insertion of anal fin to insertion of adipose fin (ANAD); 6, insertion of anal fin to top of caudal flexure (ANC1); 7, insertion of anal fin to bottom of caudal flexure (ANC2); 8, insertion of adipose fin to top of caudal flexure (ADC1); 9, insertion of adipose fin to bottom of caudal flexure (ADC2); 10, caudal flexure depth (CFD). (B) Morphological traits used in principal components analysis of hatchery and wild cutthroat and steelhead. 1, snout; 2, maxillary length; 3, head length; 4, mouth width (see Hartman 1956); 5, body depth; 6, weight (not shown); 7, caudal depth; 8, eye diameter; 9, mid-eye hypural length (MEH).



We conducted the swimming tests on fish reared under controlled conditions to prevent differential experience from biasing the results. For example, wild steelhead might outperform cutthroat in part because they rear in faster water. However, to relate our findings to wild fish, we compared the morphology of the experimental fish with that of wild fish of both species.

The morphological measurements taken on the 133 fish tested in the swimming tunnel (Table 1) were the modified truss measurements used by Taylor and Foote (1991) to compare overall body depth, body length, and depth of the caudal region used in swimming (Fig. 2A). In addition, 89 wild coastal cutthroat and 26 wild steelhead trout were collected from Mission Creek (near Belfair, Wash.) and Harvey Creek (near Arlington, Wash.) in September 1994. Morphological measurements useful in differentiating between juvenile cutthroat and steelhead in the field were taken on these fish. Although these measures did not include all of those used in the comparison of the fish tested in the swimming tunnel, they included measures of body length, body depth, and caudal depth (Fig. 2B). The hatchery-reared fish tested in the swimming tunnel were subjected to both sets of morphologic measurements to facilitate comparison with the wild fish.

The primary focus of this study was to compare the swimming performance of cutthroat, steelhead, and reciprocal hybrids. However, we considered that there might be population-specific differences so two stocks of cutthroat were used, creating eight minor cross types. Also, because of the early cutthroat mortalities, each type of pure cutthroat had two subtypes, those received as eggs and those received as fry. To determine whether we could pool minor cross types within a major cross type, and pool the subtypes of pure cutthroat (i.e., were Aberdeen and Shelton cutthroat the same, were early and late steelhead the same, were all maternal cutthroat hybrids the same regardless of the dam's stock of origin, were all paternal cutthroat hybrids the same regardless of the sire's stock of origin, and were all pure Aberdeen cutthroat or pure Shelton cutthroat the same regardless of when they were obtained), the mean critical swimming velocities were compared using two sample *t* tests. After pooling types based on the *t* tests, mean critical velocities were compared among the remaining cross types using analysis of variance (ANOVA) followed by Fisher's least significant difference test. Fisher's test compares the ranked means of adjacent pairs with the minimum difference necessary to declare significant differences (Zar 1984). Correlations between swimming ability and fork length, and swimming ability and date of test, were also examined using Spearman's rank correlation coefficient.

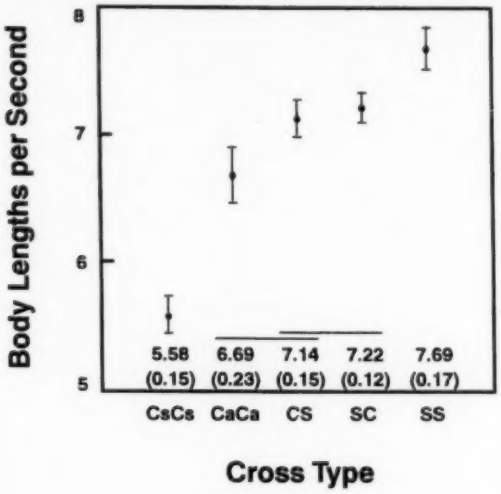
Principal components analysis with varimax rotation (Tabachnick and Fidell 1989) was used to assess body shape differences associated with swimming performance and to compare the hatchery-reared populations with those collected in the wild. Character component loadings indicate which characters are combined in each principal component. Varimax rotation rotates the component axes to maximize the variation between each principal component, thus enhancing interpretation of the data. To correct for allometric growth, all morphologic measurements were standardized to the average fork length prior to principal components analysis using the adjustment equation suggested by Ihssen et al. (1981) with Reist's (1986) adjustment coefficient:

$$M_{adj} = M_o(L/L_o)^b$$

where M_{adj} is the standardized measurement, M_o is the observed meas-

© 1996 NRC Canada

Fig. 3. Mean critical swimming velocities (bl/s) with standard error bars for pooled cross types. Values and results of Fisher's least significant difference test are given at the bottom of the graph.



urement, L is the mean fork length of the group of fish compared, L_o is the observed fork length, and b is the adjustment coefficient. The adjustment coefficient is the slope of the regression of $\log_{10}$ observed measurement on $\log_{10}$ fork length and is calculated separately for each morphological measurement. Differences in mean principal component scores among the fish types were analyzed using ANOVA followed by Tukey's test. Tukey's test was used because it allows comparisons among all groups.

Results

Swimming performance

The mean critical swimming velocities and the results of the t tests used to pool groups prior to ANOVA are given in Table 1. There were no differences in critical swimming velocities between cutthroat obtained as eggs and those obtained as fry from either hatchery (Aberdeen: $t = -0.555$, df = 11, $p = 0.59$; Shelton: $t = 0.236$, df = 19, $p = 0.81$), or between early-spawned and late-spawned steelhead ($t = -1.216$, df = 29, $p = 0.23$). Likewise, no between-population differences were found in cutthroat hybrids (maternal cutthroat: $t = 0.630$, df = 29, $p = 0.53$; paternal cutthroat: $t = 1.144$, df = 21, $p = 0.26$). However, critical swimming velocities between the two pure stocks of cutthroat differed ($t = 4.775$, df = 32, $p < 0.001$); Aberdeen cutthroat outperformed Shelton cutthroat by slightly more than 1 bl/s.

The t-test comparisons resulted in five types of fish to be tested using ANOVA: Aberdeen cutthroat (C_aC_a), Shelton cutthroat (C_sC_s), maternal cutthroat hybrids (CS), maternal steelhead hybrids (SC), and steelhead (SS). The variances of these five types were similar (Bartlett's test, $p > 0.5$). The mean critical velocities of the five types of fish differed ($F = 24.397$, df = 4,128, $p < 0.001$). Shelton cutthroat were the poorest swimmers ($U_{crit} = 5.58 \pm 0.15$ bl/s; mean ± SE), Aberdeen cutthroat were slightly better swimmers ($U_{crit} = 6.69 \pm 0.23$ bl/s), steelhead juveniles performed the best ($U_{crit} = 7.69 \pm 0.17$ bl/s), and hybrids were intermediate in swimming ability (CS, $U_{crit} = 7.14 \pm 0.15$ bl/s; SC, $U_{crit} = 7.22 \pm 0.12$ bl/s). Significant differences were identified using Fisher's least significant difference test (df = 128). No differences were found between maternal and paternal cutthroat hybrids ($p = 0.69$) nor between Aberdeen cutthroat and maternal cutthroat hybrids ($p = 0.095$). Shelton cutthroat were significantly different from Aberdeen cutthroat ($p < 0.001$) and steelhead were significantly different from paternal cutthroat hybrids ($p = 0.02$) (Fig. 3).

Table 2. Fork length (FL (mm), mean ± SD) for all fish types used in swimming tests and Spearman's rank correlation of critical swimming velocity (bl/s) with fork length and date of test, after pooling between minor cross types.

Fish type	Sample size (n)	FL	Correlation	
			U_{crit} vs. FL	U_{crit} vs. date
C_aC_a	13	88.6±7.7	–0.421	0.352
C_sC_s	23	89.6±12.0	–0.420*	–0.477*
CS	31	87.6±11.0	–0.512***	–0.513**
SC	35	87.3±13.0	–0.444**	–0.197
SS	31	100.1±9.9	0.017	–0.091
Total	133	90.9±12.2	–0.130	–0.154

Note: In all fish types the dam is listed first and subscripts indicate origin of parent: C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton.

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.005$.

There was a large range in fork length both within and among test groups (Table 2). Although the mean fork lengths differed among groups ($F = 7.002$, df = 4,128, $p < 0.001$), only the steelhead were different from the other groups (Tukey, $p < 0.05$ for all comparisons of steelhead with other test groups). Therefore, Spearman's r was calculated to test for correlation between fork length and swimming performance. The values ranged from –0.512 (CS; $p < 0.005$) to 0.017 (SS; $p > 0.50$) and were negative for all groups except steelhead. The correlation for all of the fish pooled was –0.130 ($p > 0.10$), indicating a slight tendency for smaller fish to swim faster in terms of relative speed (body lengths per second) (Table 2). Correlations between date of testing and swimming performance were also calculated because the testing period spanned 2 months. The values of Spearman's r ranged from –0.513 (CS; $p < 0.005$) to 0.352 (C_aC_a; $p > 0.20$) with the value for all of the fish pooled (–0.154, $p > 0.05$) indicating a slight tendency for fish tested early to perform better (Table 2).

Morphology

The standard errors for all of the morphological measurements taken were generally small, indicating little variation within forms (Table 3). The exception was the higher standard errors associated with the measurement of length, which is a result of the large range in size of the fish.

All measurements were size adjusted to the common fork length of the groups compared (hatchery vs. wild mean fork length = 83.91 mm, swimming tunnel fish mean fork length = 90.86 mm), so that the principal components extracted described variation in the shape of the fish. This conclusion is supported by the low values of Spearman's r calculating correlations between the principal components and fork length (Table 4).

© 1996 NRC Canada

Table 3. Morphological measurements (mean ± SE, not size adjusted) used in the principal components analyses for each type of fish measured.

	Fish type								
	C_wC_w	S_wS_w	C_aC_a	C_sC_s	C_aS_a	C_sS_a	S_aC_a	S_aC_s	SS
Sample size	89	26	13	23	9	22	15	20	31
Fork length	75.76±3.18	77.13±6.85	88.57±2.13	89.62±2.50	94.69±2.92	84.64±2.28	90.42±3.24	84.90±2.94	100.10±1.77
Snout	4.39±0.21	4.22±0.34	4.86±0.18	5.11±0.17	4.86±0.24	4.69±0.15	4.84±0.17	4.59±0.19	4.92±0.12
Maxillary	8.73±0.41	7.84±0.61	9.09±0.22	9.55±0.28	9.28±0.25	8.54±0.17	8.89±0.23	8.88±0.24	9.32±0.16
Head length	18.46±0.77	17.89±1.51	19.35±0.42	20.33±0.54	20.51±0.57	18.77±0.50	19.88±0.56	18.97±0.59	21.23±0.32
Mouth width	6.61±0.27	6.19±0.49	7.69±0.25	8.25±0.24	8.17±0.25	7.70±0.16	7.85±0.29	7.76±0.24	8.56±0.14
Body depth	14.87±0.61	16.08±1.40	18.08±0.47	19.21±0.51	19.71±0.72	17.78±0.61	18.50±0.76	17.58±0.65	20.89±0.46
Weight	6.72±1.09	8.34±2.32	8.40±0.63	9.44±0.70	10.31±0.95	8.18±0.76	9.28±0.96	7.94±0.80	12.70±0.69
Caudal depth	6.39±0.25	6.60±0.57	7.56±0.20	7.47±0.18	8.41±0.28	7.06±0.18	7.61±0.30	7.20±0.24	8.54±0.17
Eye diameter	5.25±0.18	5.51±0.34	5.49±0.14	5.45±0.11	6.06±0.11	5.58±0.10	5.84±0.17	5.79±0.17	6.36±0.09
Mid-eye-hypural	62.28±2.67	64.24±5.87	74.24±1.90	74.89±2.27	79.01±2.70	70.76±1.98	76.15±2.86	70.67±2.65	84.57±1.71
HD1			12.58±0.30	13.64±0.40	13.69±0.42	12.39±0.31	13.12±0.46	12.60±0.48	14.21±0.28
HD2			15.88±0.43	16.90±0.44	17.21±0.59	15.64±0.48	16.23±0.58	15.66±0.54	17.91±0.36
PELVDF			17.80±0.55	18.66±0.47	19.50±0.59	17.04±0.56	18.39±0.78	17.08±0.65	20.78±0.47
PELVAD			26.12±0.78	26.31±0.85	27.00±1.23	25.21±0.86	25.67±1.43	24.60±0.88	29.38±0.68
ANAD			13.24±0.35	13.70±0.40	15.19±0.44	13.42±0.53	14.06±0.68	13.10±0.51	15.86±0.36
ANC1			21.98±0.77	22.02±0.67	23.81±0.57	20.78±0.58	22.21±0.92	20.82±0.86	24.80±0.47
ANC2			18.99±0.50	19.91±0.60	20.92±0.45	18.89±0.52	19.84±0.96	19.24±0.72	22.27±0.44
ADC1			13.97±0.47	13.60±0.40	15.84±0.50	13.07±0.37	14.51±0.53	13.12±0.50	16.10±0.34
ADC2			15.87±0.44	16.08±0.47	17.70±0.52	15.16±0.38	16.58±0.68	15.41±0.64	18.85±0.35
CFD			7.07±0.21	6.83±0.18	7.75±0.25	6.75±0.16	7.28±0.26	6.90±0.20	8.10±0.18

Note: The truss measurements (see Fig. 2A) were not taken on the wild fish. All measurements are in millimetres except weight, which is in grams.

Table 4. Spearman's rank correlation of principal components with fork length (after size adjustment of traits).

Comparison	PC1	PC2	PC3	PC4
Hatchery–wild	−0.178*	0.294***	−0.037	−0.020
Swimming fish	−0.041	0.234**	−0.065	0.056

Note: Hatchery–wild indicates comparison of hatchery-reared cutthroat and steelhead with wild cutthroat and steelhead. Swimming fish indicates comparison of the five major groups tested in the swimming tunnel.

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

In the comparison of morphology among the seven types of fish tested in the swimming tunnel (C_aC_a, C_sC_s, C_aS_a, C_sS_a, S_aC_a, S_aC_s, and SS) using the modified truss measurements, four principal components (PC1–PC4) accounted for 68.58% of the variation. In general, PC1 summarized the depth of the fish forward of the dorsal fin, PC2 summarized the length of the caudal region, PC3 summarized the length of the fish behind the dorsal fin, and PC4 summarized the depth of the caudal region. The rotated component loadings and the mean component scores for each type of fish tested are shown in Table 5. PC2 (caudal region length) and PC4 (caudal region depth) were analyzed to compare the seven types because this part of the body is used for propulsion (Taylor and McPhail 1985). These components varied among types (PC2: $F = 4.92$, df = 6,126, $p < 0.001$; PC4: $F = 3.03$, df = 6,126, $p = 0.008$). In general, both Aberdeen and Shelton cutthroat had narrower caudal regions than steelhead or any of the hybrid types, although only steelhead and Shelton cutthroat differed significantly using Tukey's tests. Hybrids, with the exception of maternal Aberdeen cutthroat, tended to be intermediate (Fig. 4). When length of the caudal region was analyzed, Aberdeen cutthroat and all fish with Shelton cutthroat parents (C_sC_s, C_sS_a, and S_aC_s) had shorter caudal regions. Maternal Aberdeen cutthroat were again the hybrid exception, with longer caudal regions than any other type. Tukey's tests indicated significant differences between both steelhead and hybrids with Aberdeen cutthroat mothers and all fish with Shelton cutthroat parents (Fig. 4). Additionally, there was a positive correlation (Spearman's $r = 0.227$, $n = 133$, $p < 0.01$) between critical swimming velocity and size-adjusted caudal depth, indicating that fish with deeper caudals for their size attained higher critical swimming velocities.

In the comparison of the cutthroat and steelhead hatchery populations with wild fish using the field identification measurements, four principal components (PC1–PC4) accounted for 79.67% of the morphological variation. PC1 summarized variation in head size, PC2 summarized body and caudal depth, PC3 summarized eye size, and PC4 summarized mid-eye-hypural (MEH) length. The rotated component loadings as well as the mean component scores for the four types of fish are shown in Table 6. PC3 was not used in the comparison of hatchery and wild fish because eye size was not expected to affect swimming ability. The wild fish were generally more fusiform than the hatchery fish. Figure 5 depicts the relationships among the five groups along PC1, PC2, and PC4. In general, although the hatchery populations tended to group together, separately from the wild populations, the relationship between cutthroat and steelhead within a rearing type was consistent. Cutthroat had longer and wider heads, longer MEH measurements, and slimmer bodies than steelhead. ANOVA detected differences in each of the three principal components

© 1996 NRC Canada

Table 5. Rotated character component loadings (used to determine which characters comprise each principal component) and group mean component scores for the principal components analysis of morphological variation comparing the seven groups of cutthroat, hybrids, and steelhead tested in the swimming tunnel (see Fig. 2A for definition of characters).

(A) Rotated component loadings

Character	Fig. 2A No.	Rotated component loadings PC1	PC2	PC3	PC4
HD2	2	0.898	−0.079	0.113	0.066
PELVDF	3	0.807	0.169	0.018	0.169
HD1	1	0.759	−0.252	0.051	−0.134
ADC1	8	−0.088	0.864	0.023	−0.111
ADC2	9	−0.070	0.797	−0.044	0.259
PELVAD	4	0.050	−0.314	0.772	0.016
ANC2	7	0.076	0.384	0.727	0.026
CFD	10	−0.040	0.026	−0.097	0.901
ANAD	5	0.223	0.093	0.392	0.564
ANC1	6	0.040	0.495	0.447	0.024
% variation		21.08	19.82	15.06	12.62

(B) Mean component scores

Group	Mean component scores PC1	PC2	PC3	PC4
C_aC_a	−0.548	−0.270	−0.092	−0.226
C_sC_s	0.862	−0.345	0.085	−0.667
C_aS_a	0.005	0.842	−0.312	0.391
C_sS_s	−0.056	−0.445	0.541	0.053
S_aC_a	−0.175	0.216	−0.565	0.023
S_aC_s	0.039	−0.282	0.260	0.100
SS	−0.312	0.519	−0.212	0.363

Note: The percentage of total variation explained by each component is given below the character component loadings. In all fish types the dam is listed first and subscripts indicate origin of parent. C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton.

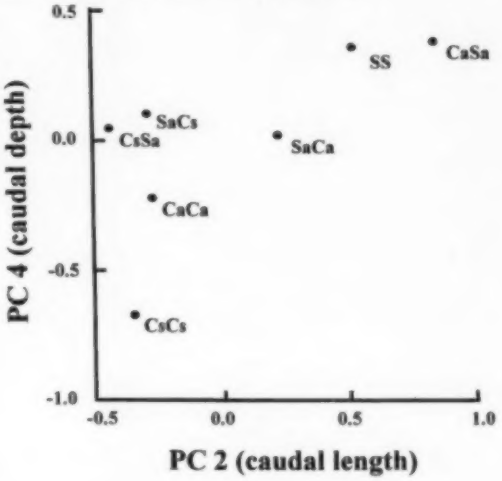
examined (PC1: $F = 17.76$, df = 4,177, $p < 0.001$; PC2: $F = 12.84$, df = 4,177, $p < 0.001$; PC4: $F = 5.46$, df = 4,177, $p < 0.001$). However, Tukey's tests comparing fish within a rearing type only identified significant differences between wild cutthroat and steelhead in PC1 (head size) and between hatchery steelhead and Shelton cutthroat in PC1 (head size).

Discussion

Steelhead of hatchery origin were capable of higher critical swimming velocities than cutthroat of hatchery origin when reared in the same environment. The magnitude of this difference in mean critical swimming velocity ranged from 2.21 bl/s for steelhead and Shelton cutthroat to 1 bl/s for steelhead and Aberdeen cutthroat. These differences in swimming ability are consistent with differences in juvenile and adult habitat utilization and ocean migrations. Steelhead attain higher critical swimming velocities, are generally found in faster water in the streams as juveniles, and undergo more extensive migrations than cutthroat.

The morphological analysis showed that the differences in swimming ability were likely associated with heritable differences in body shape between the species and not differences

Fig. 4. Plot of mean principal components scores for PC2 (caudal length) and PC4 (caudal depth) derived from morphological analysis of cross types tested in swimming tunnel. In all fish types the dam is listed first and subscripts indicate origin of parent. C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton.



in hatchery practices. Cutthroat tended to have shorter and narrower caudal regions than steelhead, features poorly suited to sustained swimming at high velocities (Taylor and McPhail 1985; Taylor and Foote 1991). Furthermore, although the juvenile cutthroat and steelhead collected in the wild were generally more fusiform than the hatchery fish (possibly indicating increased swimming ability; Thomas and Donahoo 1977; Swain et al. 1991), the shape relationship between the species was consistent within rearing environments. Cutthroat from either hatchery or from the two streams had longer heads and narrower caudal regions than steelhead from the respective rearing environments. Therefore, the differences in swimming ability between cutthroat and steelhead in this study are relevant to at least some populations of wild fish. Wild cutthroat should exhibit lower critical swimming velocities than wild steelhead from the same region, but variation among populations exists (Thomas and Donahoo 1977; Tsuyuki and Williscroft 1977; the present study).

The difference in swimming ability between the two stocks of cutthroat mirrored their morphology. Both sets of morphological measurements (modified truss and field identification) identified the Shelton cutthroat as having deeper and larger heads and deeper midbodies than any of the other fish types, including Aberdeen cutthroat. This was especially evident when head and body depth were compared with caudal region development. Shelton cutthroat had significantly poorer swimming ability and had a body shape least suited to sustained swimming. Although the difference was not significant, hybrids with Shelton parents (maternal or paternal) also tended to have poorer swimming ability than hybrids with Aberdeen parents. This would seem to indicate an influence on swimming ability associated with stock differences in body shape. It is possible that the increased swimming ability and more fusiform morphology of fish with Aberdeen cutthroat parents

© 1996 NRC Canada

Table 6. Rotated character component loadings (used to determine which characters comprise each principal component) and group mean component scores for the principal components analysis of morphological variation comparing hatchery and wild juvenile cutthroat and steelhead.

(A) Rotated component loadings

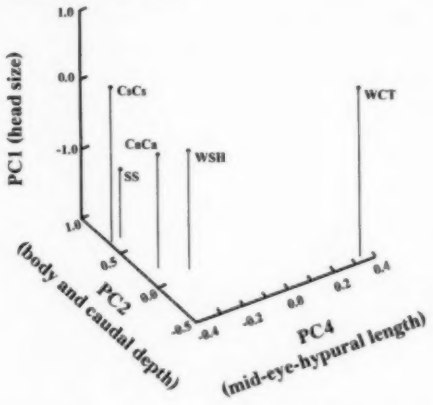
Character	Fig. 2B No.	Rotated component loadings PC1	PC2	PC3	PC4
Snout	1	0.878	−0.016	−0.058	0.100
Maxillary	2	0.867	−0.153	0.156	0.153
Head length	3	0.738	−0.128	0.327	0.346
Mouth width	Not shown	0.593	0.434	−0.501	0.044
Body depth	5	−0.021	0.868	−0.039	−0.153
Weight	Not shown	−0.124	0.817	−0.199	−0.284
Caudal depth	7	−0.045	0.746	0.166	0.208
Eye diameter	8	0.166	0.048	0.915	0.019
Mid-eye-hypural	9	−0.299	0.099	0.006	−0.896
% variation		28.38	24.64	14.36	12.29

(B) Mean component scores

Group	Mean component scores PC1	PC2	PC3	PC4
C_aC_a	−0.355	0.198	−0.743	−0.329
C_aC_s	0.247	0.638	−1.111	−0.384
SS	−1.010	0.654	−0.218	−0.340
C_wC_w	0.416	−0.442	0.187	0.336
S_wS_w	−0.260	0.071	0.976	−0.240

Note: The percentage of total variation explained by each component is given below the character component loadings. In all fish types the dam is listed first and subscripts indicate origin of parent. C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton; w, wild.

Fig. 5. Plot of mean principal components scores for PC1 (head size), PC2 (body and caudal depth) and PC4 (mid-eye-hypural length) derived from morphological analysis of hatchery and wild cutthroat and steelhead. In all fish types the dam is listed first and subscripts indicate origin of parent. C, cutthroat; S, steelhead; a, Aberdeen; s, Shelton; w, wild.



are due to steelhead genetic influence in the Aberdeen cutthroat stock.

Generally, cutthroat–steelhead hybrids had intermediate swimming abilities. The hybrid trout had mean critical swimming velocities closer to those of steelhead regardless of whether they had a maternal or paternal steelhead parent. There were no significant differences in swimming ability found among any of the hybrid types. The morphology of the hybrid trout was also generally intermediate to those of the parental species. Hybrids with Aberdeen cutthroat mothers were exceptional in both caudal length and depth, with mean component scores higher than all other fish types. These fish also outperformed the other hybrid types in the swimming tests, again indicating the strong association between caudal region development and swimming ability.

Brett (1965) indicated that although larger fish attain higher absolute sustained swimming speeds, they exhibit reduced swimming ability relative to their size (body lengths per second). Results from this study agree with Brett's results, except for steelhead; smaller fish performed better in the swimming tests in terms of relative speed (body lengths per second). This difference between steelhead and the other fish types tested is possibly due to differences in the morphology of the fish and how well suited they are to sustained swimming. Because of allometric growth in cutthroat and steelhead, the relative shape of the fish changes with fork length and therefore species differences become more pronounced in larger fish. For example, it is very difficult to differentiate between juvenile cutthroat and steelhead less than 50 mm in length, yet it is relatively easy to distinguish them as adults. These allometric shape changes could be responsible for the fact that steelhead swimming ability improved with size whereas the other fish types became somewhat poorer swimmers with size. As a steelhead grows it rears in faster water and matures into a fish that undergoes extensive marine migrations, whereas cutthroat do not. It has been shown (Winans and Nishioka 1987) that there are changes in the shape of the caudal region of coho during the period of development associated with migration to salt water. However, further studies comparing the morphology of adult steelhead and cutthroat would need to be carried out to test this hypothesis.

The trend for fish tested earlier to perform better in the swimming tests is possibly due to the effects of a confined rearing environment on swimming ability. The fish continued to grow throughout the testing period and were kept in the same containers to minimize differences among types caused by environment.

The main purpose of this work was to assess the swimming ability and related morphology of cutthroat, steelhead, and hybrids in an attempt to address the relative performance and therefore the potential effects of occasional hybridization between coastal cutthroat and steelhead. It is unknown whether natural hybrid progeny assume a cutthroat, steelhead, or some intermediate life history, or to what age they survive. Previous work on cutthroat–steelhead hybrids (Campton 1981; Campton and Utter 1985) suggested that the potential for decreased fitness of the hybrids existed, because only young-of-the-year hybrids were detected. Furthermore, they were unable to positively determine if backcross or F_2 hybrids were present using protein allozymes. Swimming ability has fitness consequences both in terms of vulnerability to predation (Bams 1967) and in relation to the energetic demands of migration (Tsuyuki and Williscroft 1977; Taylor and McPhail 1985). Therefore, comparison of swimming ability could yield insights into the rela-

© 1996 NRC Canada

tive viability of the hybrids and parental cutthroat and steelhead. Hybrid trout were intermediate in both swimming ability and morphology, putting them at a competitive disadvantage when compared with steelhead in fast water. However, all of the hybrid types tested demonstrated superior swimming ability and morphology better suited to sustained swimming than cutthroat. Habitat use by juvenile cutthroat is generally thought to be dictated by competitive displacement by coho and steelhead and a lack of morphological adaptation to either fast or slow water (Bisson 1988). In streams containing all three salmonids, cutthroat often occupy less desirable, intermediate habitat. However, in the absence of coho, cutthroat move into deeper, slower water (Johnston 1982; Bisson et al. 1988; Pauley et al. 1989). Consequently, the relative competitiveness of hybrid and cutthroat trout based on swimming ability and related morphology could be influenced by the presence of other salmonids. In a stream without coho, hybrids might occupy the intermediate habitat, cutthroat might reside in pools, and steelhead might live in riffles. In this case, the hybrids might be at a competitive disadvantage, analogous to cutthroat in a stream containing coho and steelhead. However, in a stream containing coho, the competitive outcome is less clear. If cutthroat and hybrids competed for the intermediate habitat, the superior swimming ability of the hybrids might put them at a competitive advantage. Therefore, this comparison of relative swimming ability does not demonstrate hybrid depression. There is the potential for a competitive advantage when cutthroat–steelhead hybrids are compared with cutthroat. However, swimming ability is just one measure of performance and further studies comparing other traits, such as aggression and habitat preference, will be important in predicting the viability of hybrids relative to the parental species.

In summary, juvenile steelhead achieved higher critical swimming velocities than cutthroat and hybrids were intermediate in both swimming performance and morphology. Depending on the life history adopted by hybrids, there is the potential for a competitive advantage over cutthroat. This advantage of the F_1 progeny could then contribute to introgression between coastal cutthroat and steelhead.

Acknowledgments

We thank Peter Hawkins, Andrew Hendry, Jay Hensleigh, Bert Lewis, Kelly Moore, and Glenn Yokoyama for assistance in rearing the fish; Michael Kinnison for help in carrying out the swimming performance tests; and Bob Paulsen and John Kugen from the Aberdeen hatchery and Larry Barger from the Shelton hatchery for their cooperation in obtaining the gametes to establish the families. We also thank Drs. C.J. Foote and E.B. Taylor for assistance in the analysis and writing. Andrew Hendry drew Fig. 2 and critically reviewed the paper, as did M. Kinnison, Dr. G. Winans, and Dr. W.K. Hershberger. Finally, we thank Dr. P. Bentzen, P. Hawkins, A. Hendry, M. Kinnison, S. Miller, K. Moore, and A. Spidle for assistance in the field. This research was supported by the H. Mason Keeler Endowment.

References

Arnold, M.L., and Hodges, S.A. 1995. Are natural hybrids fit or unfit relative to their parents? Trends Ecol. Evol. **10**: 67–71.

Bams, R.A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. J. Fish. Res. Board Can. **24**: 1117–1153.

Behnke, R.J. 1970. The application of cytogenetic and biochemical systematics to phylogenetic problems in the family Salmonidae. Trans. Am. Fish. Soc. **99**: 237–248.

Behnke, R.J. 1972. The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Board Can. **29**: 639–671.

Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Md.

Bisson, P.A., Sullivan, K., and Nielsen, J.L. 1988. Channel hydraulics, habitat use, and body form of juvenile coho salmon, steelhead, and cutthroat trout in streams. Trans. Am. Fish. Soc. **117**: 262–273.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. **21**: 1183–1226.

Brett, J.R. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. **22**: 1491–1501.

Burgner, R.L., Light, J.T., Margolis, L., Okazaki, T., Tautz, A., and Ito, S. 1992. Distribution and origins of steelhead trout (*Oncorhynchus mykiss*) in offshore waters of the North Pacific Ocean. Int. North Pac. Fish. Comm. Bull. **51**: 1–92.

Busack, C.A., and Gall, G.A.E. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). Can. J. Fish. Aquat. Sci. **38**: 939–951.

Campton, D.E. 1981. Genetic structure of sea-run cutthroat trout (*Salmo clarki clarki*) populations in the Puget Sound area. M.S. thesis, University of Washington, Seattle, Wash.

Campton, D.E., and Utter, F.M. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. Can. J. Fish. Aquat. Sci. **42**: 110–119.

Carpenter, L.T. 1987. A comparative study of short-term swimming performance in fry of five salmonid species at different temperatures. M.S. thesis, University of Washington, Seattle, Wash.

Chevassus, B. 1979. Hybridization in salmonids: results and perspectives. Aquaculture, **17**: 113–128.

Dowling, T.E., and Childs, M.R. 1992. Impact of hybridization on a threatened trout the southwestern United States. Conserv. Biol. **6**: 355–364.

Flagg, T.A. 1981. Swimming stamina and survival related to swimming fatigue in response to direct seawater entry during the parr–smolt transformation of coho salmon (*Oncorhynchus kisutch*). M.S. thesis, University of Washington, Seattle, Wash.

Foote, C.J., and Larkin, P.A. 1988. The role of male choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). Behaviour, **106**: 43–62.

Giger, R.D. 1972. Ecology and management of coastal cutthroat trout in Oregon. *In* Fisheries research report No. 6. Oregon State Game Commission, Portland, Oreg. pp. 1–43.

Gold, J.R. 1977. Systematics of western North American trout (*Salmo*), with notes on the redband trout of Sheepheaven Creek, California. Can. J. Zool. **55**: 1858–1873.

Halliburton, R., Pipkin, R.E., and Gall, G.A.E. 1983. Reproductive success of artificially hybridized golden trout (*Salmo aguabonita*) and rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **40**: 1264–1269.

Hammar, J., Dempson, J.B., and Verspoor, E. 1991. Natural hybridization between Arctic char (*Salvelinus alpinus*) and brook trout (*S. fontinalis*): evidence from northern Labrador. Can. J. Fish. Aquat. Sci. **48**: 1437–1445.

Hartman, G.F. 1956. A taxonomic study of cutthroat trout, *Salmo clarki clarki* Richardson, rainbow trout *Salmo gairdneri* Richardson and reciprocal hybrids. M.A. thesis, University of British Columbia, Vancouver, B.C.

© 1996 NRC Canada

Hubbs, C.L. 1955. Hybridization between fish species in nature. Syst. Zool. **4**: 1–20.

Ihssen, P.E., Evans, D.O., Christie, W.J., Reckahn, J.A., and Desjardine, R.L. 1981. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes Region. Can. J. Fish. Aquat. Sci. **38**: 1790–1807.

Johnson, O.W., Waples, R.S., Wainwright, T.C., Neely, K.G., Waknitz, F.W., and Parker, L.T. 1994. Status review for Oregon's Umpqua River sea-run cutthroat trout. NOAA Tech. Memo. No. NMFS-NWFSC–15.

Johnston, J.M. 1982. Life histories of anadromous cutthroat with emphasis on migratory behavior. *In* Salmon and trout migratory behavior symposium. *Edited by* E.L. Brannon and E.O. Salo. School of Fisheries, University of Washington, Seattle, Wash. pp. 123–127.

Johnston, J.M., and Mercer, S.P. 1976. Sea-run cutthroat in saltwater pens: broodstock development and extended juvenile rearing (with a life history compendium). *In* Fisheries research report No. AFS–57. Washington State Game Department, Olympia, Wash.

Jordan, W.C., and Verspoor, E. 1993. Incidence of natural hybrids between Atlantic salmon, *Salmo salar* L., and brown trout, *Salmo trutta* L., in Britain. Aquacult. Fish. Manage. **24**: 373–377.

Leary, R.F., Allendorf, F.W., Phelps, S.R., and Knudsen, K.L. 1987. Genetic divergence and identification of seven cutthroat trout subspecies and rainbow trout. Trans. Am. Fish. Soc. **116**: 580–587.

Mayr, E. 1963. Populations, species, and evolution. Belknap Press, Cambridge, Mass.

Moser, M., Olson, A.F., and Quinn, T.P. 1990. Effects of dummy ultrasonic transmitters on juvenile coho salmon. Am. Fish. Soc. Symp. **7**: 353–356.

Nehlsen, W., Williams, J.E., and Lichatowich, J.A. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho and Washington. Fisheries (Bethesda), **16**: 4–21.

Pauley, G.B., Oshima, K., Bowers, K.L., and Thomas, G.L. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates. (Pacific Northwest) sea-run cutthroat trout. U.S. Fish Wild. Serv. Biol. Rep. No. 82(11.86). U.S. Army Corps of Engineers No. TR EL–82–4.

Reist, J.D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Can. J. Zool. **64**: 1363–1368.

Smith, L.S., and Newcomb, T.W. 1970. A modified version of the Blazka respirometer and exercise chamber for large fish. J. Fish. Res. Board Can. **27**: 1321–1324.

Stearley, R.F., and Smith, G.R. 1993. Phylogeny of the Pacific trouts and salmons (*Oncorhynchus*) and genera of the family Salmonidae. Trans. Am. Fish. Soc. **122**: 1–33.

Swain, D.P., Riddell, B.E., and Murray, C.B. 1991. Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. Can. J. Fish. Aquat. Sci. **48**: 1783–1791.

Tabachnick, B.G., and Fidell, L.S. 1989. Using multivariate statistics. 2nd ed. Harper-Collins Inc., New York.

Taylor, E.B. 1986. Differences in morphology between wild and hatchery populations of juvenile coho salmon. Prog. Fish-Cult. **48**: 171–176.

Taylor, E.B., and Foote, C.J. 1991. Critical swimming velocities of juvenile sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka* (Walbaum). J. Fish Biol. **38**: 407–419.

Taylor, E.B., and McPhail, J.D. 1985. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. **42**: 2029–2033.

Thomas, A.E., and Donahoo, M.J. 1977. Differences in swimming performance among strains of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **34**: 304–306.

Trotter, P.C. 1987. Cutthroat native trout of the west. Colorado Associated University Press, Boulder, Colo.

Trotter, P.C. 1989. Coastal cutthroat trout: a life history compendium. Trans. Am. Fish. Soc. **118**: 463–473.

Tsuyuki, H., and Williscroft, S.N. 1977. Swimming stamina differences between genotypically distinct forms of rainbow (*Salmo gairdneri*) and steelhead trout. J. Fish. Res. Board Can. **34**: 996–1003.

Utter, F.M. 1981. Biological criteria for definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973. Can. J. Fish. Aquat. Sci. **38**: 1626–1635.

Utter, F.M., and Allendorf, F.W. 1994. Phylogenetic relationships among species of *Oncorhynchus*: a consensus view. Conserv. Biol. **8**: 864–867.

Verspoor, E. 1988. Widespread hybridization between native Atlantic salmon (*Salmo salar*) and introduced brown trout (*S. trutta*) in eastern Newfoundland. J. Fish Biol. **32**: 327–334.

Wilson, G.M., Thomas, W.K., and Beckenbach, A.T. 1985. Intra- and inter-specific mitochondrial DNA sequence divergence in *Salmo*: rainbow, steelhead, and cutthroat trouts. Can. J. Zool. **63**: 2088–2094.

Winans, G.A., and Nishioka, R.S. 1987. A multivariate description of change in body shape of coho salmon (*Oncorhynchus kisutch*) during smoltification. Aquaculture, **66**: 235–245.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall Inc., Englewood Cliffs, N.J.

© 1996 NRC Canada

Spatial analysis and mapping of acoustic survey data in the presence of high local variability: geostatistical application to North Sea herring (*Clupea harengus*)

Christos D. Maravelias, David G. Reid, E. John Simmonds, and John Haralabous

Abstract: A methodology is presented for the spatial analysis and geostatistical mapping of acoustic survey data containing a small number of observations with very high values. Three different scales of spatial structures were identified: an unresolved small-scale variability that accounted for almost 32% of variance, and two structure components of 6.5 (52%) and 20 (16%) nautical miles. The destabilizing effect that the few high values had when computing the variogram was dealt with by using the robust variogram estimator, which effectively helped to reveal nested structures and adjust autocorrelation ranges through the best fit model. The correct proportion of the local small-scale unresolved variability, the nugget effect, was accurately estimated using cross-validation iterating fitting techniques. The optimal variogram is determined as that which minimizes the cross-validation mean square errors. Maps are generated from a range of possible variograms and the relative value of each is discussed in the light of the ecological behaviour of the stock. The technique is valuable for situations where fish distributions are highly aggregated and survey data are skewed owing to few but representative high values that can represent a large biomass and are often characteristic and repeatable for a particular stock.

Résumé : Nous présentons une technique pour l'analyse spatiale et la cartographie géostatistique des données acoustiques qui contiennent un petit nombre de valeurs très élevées. Nous avons identifié trois échelles différentes de structures spatiales : une variabilité non résolue à petite échelle qui représentait près de 32% de la variance, et deux composantes de 6,5 (52%) et 20 (16%) milles marins. Pour contrer l'effet déstabilisant des quelques valeurs élevées sur le calcul du variogramme, nous avons eu recours à l'estimateur robuste du variogramme qui a efficacement fait ressortir des structures emboîtées et aidé à ajuster les plages d'autocorrélation par le biais du modèle du meilleur ajustement. La proportion correcte de variabilité locale non résolue à petite échelle, l'effet de « pépite, » a été estimée avec précision à l'aide de techniques d'ajustement itératif avec validation croisée. Le variogramme optimal est celui qui réduit au minimum les erreurs quadratiques moyennes de la validation croisée. Les cartes sont produites à partir d'une gamme de variogrammes possibles, et la valeur relative de chacune est analysée au regard du comportement écologique du stock. La technique est intéressante dans les situations où les distributions des poissons sont fortement regroupées et où les données des relevés sont asymétriques à cause de valeurs élevées, peu nombreuses mais représentatives, qui peuvent représenter une forte biomasse et sont souvent caractéristiques d'un stock donné et reproductibles.
[Traduit par la Rédaction]

Introduction

Spatial dependence or continuity is a common phenomenon in marine ecology; organisms tend to have patchy distributions and are found in high densities in some areas and are sparse or absent in others. Marine species, including fish, are often concentrated in response to a particular feature of the marine environment, i.e., cliff, current, shelf edge, or reef. Fish species tend to be organised in structures, hence their distribution is not random, either in space or in time. The range of these structures varies dramatically, from a few metres (e.g., schools) to many kilometres. Geostatistics is a branch of applied statistics that focuses on the detection, modelling, and estimation of spatial patterns (Rossi et al. 1992).

Fisheries acoustics surveys perform continuous sampling along a cruise track. As a result, the data are serially autocorrelated; that is, data points that are close together are usually more correlated than those that are further apart. Additionally, acoustic surveys are usually carried out using regular grid patterns, and hence they are rarely adaptable to classical estimation methods and random sampling theory. Hence, in the light of the the above observations, the application of geostatistical methods would seem to be appropriate to acoustic survey data.

A geostatistical analysis typically involves three steps. The first step is to describe the spatial structures of the studied variable through the so-called structure functions, which allow us to quantify the spatial dependency and partition it along the various distance classes. The most commonly used function is the variogram (Matheron 1971). The variogram gives a precise

Received January 5, 1995. Accepted January 29, 1996.
J12703

C.D. Maravelias,[1] D.G. Reid, and E. J. Simmonds. Scottish Office of Agriculture, Environment and Fisheries Department (SOAFD), Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen AB11 9DB, Scotland, U.K.
J. Haralabous. Institute of Marine Biology of Crete, P.O. Box 2214, Heraklion, Crete, Greece.

[1] Author to whom all correspondence should be addressed.
e-mail: maraveliasc@marlab.ac.uk

© 1996 NRC Canada

content to the traditional concept of the influence zone of a sample. The more or less rapid increase of the variogram represents the more or less rapid deterioration of the influence of a given sample over more and more remote zones of the deposit (Matheron 1963). The second step is the fit of a statistical model, such as linear, spherical, or exponential, which allows us to relate the observed structure to hypothesized generating processes. The third step is the application of a linear method of spatial prediction known as kriging (Matheron 1971). The technique uses the variogram to define coefficients to weight the observations taken over an area to obtain spatially interpolated, or kriged, estimates of abundance at any point of this area. These estimated values can then be used either locally for mapping the studied variable, in this case fish density (Simard et al. 1992, 1993), or over the whole area for what is known as global estimation (Sullivan 1991; Simard et al. 1992, 1993).

Although the kriging weights and the consequent variance of the kriging estimates are based on the chosen model, published work on geostatistics, while stressing the importance of the variogram, usually underplays the problems in estimating it (Cressie and Hawkins 1980).

One of these problems is the complex spatial inhomogeneities that are often seen in fish distribution data, and especially acoustic survey data. They usually include a small number of extremely high values. These values may represent significant portions of the total biomass, but a disproportionately small proportion of the total number of samples. They are often characteristic of particular stocks and are seen during every survey of such stocks. They cannot be considered as sampling errors or statistical outliers. Their presence will eventually lead to highly skewed (erratic) variograms (i.e., nonrobust statistics).

A common problem in geostatistics is to fit the variogram model especially near the origin (first 3–5 points). The behaviour at the origin characterizes the continuity of the studied variable and plays a crucial role in the fitting of a variogram model because it has a tremendous influence on the krigged estimate of abundance throughout an area. There are four classical types of behaviour at the origin (Matheron 1971): parabolic trend, linear behaviour, nugget effect (discontinuity at the origin), and pure nugget effect (completely random, flat variogram). At the scale of a dozen or a hundred metres, a phenomenon that has, for instance, a range of the order of centimetres is no longer apparent on the experimental variogram, except as a discontinuity at the origin, the nugget effect (Matheron 1971). Therefore, the nugget is usually due to a spatial variability below the minimum lag distance, which cannot be modelled with the present sampling design, but it can also be due to experimental error, which adds variance to the estimation. Previous studies have shown that fisheries acoustic data usually exhibit an apparent discontinuity at the origin, a nugget effect (Sullivan 1991 and references therein). This unresolved small-scale variability (nugget) can constitute a large proportion of the overall variance. Although several successful attempts were made to apply geostatistics to the estimation and mapping of fisheries acoustics data (Conan et al. 1994; Sullivan 1991; Simard et al. 1992, 1993), little attention has been paid to correctly estimating the proportion of the variance included as nugget when fitting the variogram, and thus including it correctly when mapping a resource with (*i*) a positive highly skewed distribution, (*ii*) a small number of high value observations, and (*iii*) a very fine medium- and large-scale variability such as a schooling fish population.

In this paper we examined acoustic data collected during the 1992 International Council for the Exploration of the Sea (ICES) (division IVa) coordinated herring acoustic survey. The North Sea herring population that aggregates in patches of dense schools over a wide area of continental shelf was used here as an example. It is a schooling population with dense schools with typical dimensions of tens to a few hundreds of metres, giving a large proportion of fine-scale variability. The schools appear to aggregate in groups that may be linked with oceanographic features on scales of 5–20 km, giving some medium-scale spatial correlation. These patches are found over an area that extends over most of the northern North Sea but is concentrated within an area of about 300 by 600 km. Geostatistical methodology was applied to analyse the spatial distribution and evaluate the range of autocorrelation, the shape of the variogram, the sill level, and the relative importance of the variability at various scales.

The main aim of the present study was to find a geostatistical methodology that was appropriate to the type of highly skewed data that is common in fisheries acoustics and to produce the most appropriate map of the fish distribution. The disturbing effect that the small number of high values had when computing the variogram was dealt with by using the robust variogram estimator, which effectively helped to reveal nested structures and adjust autocorrelation ranges. The correct proportion of the nugget effect was accurately adjusted using cross-validation iterating fitting techniques. The utilization of this information in the consequent kriging enabled much more realistic maps to be generated and spatial patterns to be described.

Materials and methods

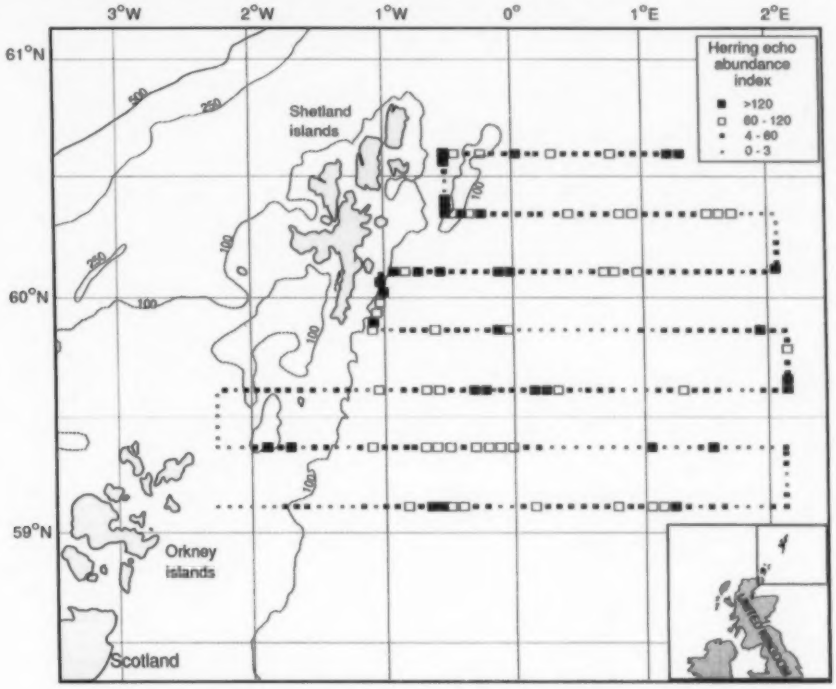
Acoustic survey

The survey was carried out on prespawning concentrations of autumn-spawning herring from 13 to 31 July, 1992, using the fisheries research vessel FRV *Scotia*. We used a Simrad EK500 38-kHz sounder and echo-integrator. Further data analysis was carried out using Simrad BI500 and Marine Lab Analysis systems. The survey track was selected to cover the area in a single level of sampling intensity based on the limits of herring densities found in the previous years. A transect spacing of 15 nautical miles (n.miles; 1 n.mile = 1852 m) was used (Fig. 1). The ends of the tracks were positioned at half the actual track spacing from the area boundary, giving equal track length in any rectangle within the area. The between-track data could then be included in a conventional data analysis for an unbiased estimate of fish abundance. In geostatistics, however, any data point with a known location can be included in the analysis. The origin of the survey grid was selected randomly within a 15-n.mile interval and laid out with systematic spacing from the random origin. Data from the echo integrator were summed over 15-min periods (2.5 n.miles at 10 knots; 1 knot = 1.852 km/h). The echo sounder provided approximately 2.5×10^8 measurements of fish density during an acoustic survey of this herring population. To allow analysis of these data sets the number of observations must be reduced. A 2.5-n.mile averaging interval was chosen for the routine surveys and was considered as an acceptable compromise between the need to reduce the volume of data and the need to retain information on the spatial structure of the population.

The echo-integrator data were collected from 9 m below the sea surface (transducer at 5 m depth) to 1 m above the seabed (details of

© 1996 NRC Canada

Fig. 1. Map of the bottom topography of the study area, east of the Shetland and Orkney islands (ICES division IVa): survey track of FRV *Scotia*, July 1992. Rectangles are herring EAI (echo-abundance index) indicators on sample locations.



equipment and survey design are reported in Simmonds et al. 1993). A starting depth of 9 m was selected to remove reflections from bubbles in the surface layer. During this survey herring were not found shallower than this depth. The integration interval was terminated 1 m above the seabed because it was important to ensure that no echos from the seabed were included in the integral. In this survey, herring schools were normally found at least a few metres clear of the seabed. For the echo sounder system used, with a pulse length of 1.0 ms and a beam width of 8°, 1 m provided an acceptable safety margin.

Herring were found mostly in water with the seabed deeper than 100 m, with traces being found in water with depths of up to 250 m. The herring density data were available on a fine scale along the survey track (every 2.5 n.miles), in the form of herring echo abundance index (EAI). Trawl hauls were carried out during the survey to identify the herring schools and to provide biological data on herring. The herring abundance index was calculated by partitioning the part of the echo-integral identified, by trawl, as herring, from the echo-integrator output, and is linearly related to herring biomass (Foote 1983).

The coordinates on the original cruise track data were expressed as degrees of longitude and latitude. To use the coordinates in the data analysis, degrees were converted to nautical miles. For the purpose of the present analysis, a subsection of the survey area was used. A subsection rather than the entire area was used because (*i*) the survey was stopped for 2 days and the inclusion of additional samples would have introduced temporal variation in the analysis, (*ii*) the older year-classes were more likely to be found east of the Shetland Islands, while younger year-classes were more likely to be found west of Shetlands, hence it constituted a biologically homogeneous area, and (*iii*) it comprised an ecologically homogeneous area, as the area east of the Shetlands is quite different from the area west of the Shetlands in terms of the hydrography and topography.

Structural analysis and variogram estimation

The two numerical values (here, representing herring densities) $Z(x)$ and $Z(x + \mathbf{h})$ at two points x and $x + \mathbf{h}$ are separated by the vector $\mathbf{h}$.Then the variability between these two quantities is characterized by the variogram function $2\gamma(x,\mathbf{h})$, which is defined (Journel and Huilbregts 1978) as the expectation of the random variable, $(Z(x) - Z(x + \mathbf{h}))^2$, i.e.,

$$2\gamma(x,\mathbf{h}) = E((Z(x) - Z(x + \mathbf{h}))^2)$$

In the present analysis, second-order stationarity is assumed (Journel and Huilbregts 1978, Chap. 2), where (*i*) the mathematical expectation (or mean) of the random function, $E(Z(x))$, exists and does not depend on the support point, x:

$$E(Z(x)) = m, \forall x$$

and (*ii*) for each pair of regionalized variables $(Z(x) - Z(x + \mathbf{h}))$ the covariance, C, exists and depends on the separation distance, $\mathbf{h}$,

$$C(h) = E(Z(x + \mathbf{h})\ Z(x)) - m^2, \forall x$$

(for a definition and a complete presentation of the theory of regionalized variables and intrinsic random functions see Matheron 1971; Journel and Huilbregts 1978). The stationarity of the covariance implies the stationarity of the variogram (Journel and Huilbregts 1978).

Under the intrinsic hypothesis (which is simply the second-order stationarity of the differences, $Z(x) - Z(x + \mathbf{h})$, the variogram function depends only on the separation vector (modulus and direction) and not on the location, x. According to Journel and Huilbregts (1978, p. 11) it is then possible to estimate the variogram $2\gamma(\mathbf{h})$ from the available data: an estimator $2\gamma^*(\mathbf{h})$ is the arithmetic mean of the squared differences between two experimental measures, $Z(x_i) - Z(x_i + \mathbf{h})$, at any two points separated by the vector $\mathbf{h}$, i.e.,

© 1996 NRC Canada

$$2\gamma^*(\mathbf{h}) = \frac{1}{N(\mathbf{h})}\sum_{i=1}^{N(\mathbf{h})}(Z(x_i) - Z(x_i+\mathbf{h}))^2$$

where $N(\mathbf{h})$ is the number of experimental pairs $Z(x_i) - Z(x_i+\mathbf{h})$ of data separated by the vector $\mathbf{h}$.

The presence of extremely high values (often called outliers) can seriously distort and mask the real variogram patterns (Krige and Magri 1982). Thus, a procedure is needed that can lead to a better definition of the variogram pattern and parameters. In the analysis to follow we used the robust estimator of the variogram, developed by Cressie and Hawkins (1980), in an attempt to compute variograms that are less influenced by the disturbing effects of very high values:

$$2\gamma^*(\mathbf{h}) = \left(\frac{1}{N(\mathbf{h})}\sum_{i=1}^{N(\mathbf{h})}|Z(x_i) - Z(x_i+\mathbf{h})|^{1/2}\right)^4 \Big/ \left(0.457 + \frac{0.494}{|N(\mathbf{h})|}\right)$$

This estimator is robust to contamination by outliers (for an application see also Sullivan 1991). As described in Cressie (1991) for handling outliers, this method greatly enhanced the variogram continuity.

Variograms can be computed as either an average over all directions in which case the lag measure is scalar and hence called omnidirectional, or specific to a particular direction in which case the lag measure is a vector, and called directional (Rossi et al. 1992). In geostatistics the situation where a variogram exhibits a longer range (i.e., better correlation) in one direction than another is called anisotropy (in the case where the ranges in every direction are similar the situation is called isotropy). Directional variograms were also computed and compared using latitudinal distances versus longitudinal distances across the sample area. Because ranges were found to be similar regardless of orientation, no significant large-scale anisotropy was evident. Hence, the variogram behaviour was assumed to be the same in all directions, and results presented correspond to isotropic (omnidirectional) variograms. Experimental omnidirectional variograms were computed and applied to the raw data.

Variogram model fitting

To characterize the spatial structure of the studied variable, the construction of a model was essential. Two of the three basic isotropic models were utilized here: the spherical and the exponential (the third is the linear model, which was not applicable to the kind of stationarity that characterized our data). These two models in terms of the semivariogram are given by (Cressie 1991, p. 61)

$$\gamma(\mathbf{h};\theta) = \begin{cases} 0, & \mathbf{h} = 0 \\ c_0 + c_s\,((3/2)(\|\mathbf{h}\|/\alpha_s) - (1/2)(\|\mathbf{h}\|/\alpha_s)^3), & 0 < \|\mathbf{h}\| \le \alpha_s \\ c_0 + c_s, & \|\mathbf{h}\| \ge \alpha_s \end{cases}$$

for the spherical model, and

$$\gamma(\mathbf{h};\theta) = \begin{cases} 0, & \mathbf{h} = 0 \\ c_0 + c_e\,(1 - \exp(-\|\mathbf{h}\|/\alpha_e)), & \mathbf{h} \ne 0 \end{cases}$$

for the exponential model, where c is the sill of the variogram that represents the maximum level of variability (c_s for a spherical function and c_e for exponential function), α is the range of the variogram beyond which data are no longer autocorrelated (α_s and α_e in a similar way, for the exponential model the range is one third of the practical range $\alpha' = 3\alpha_e$), and c_0 is the nugget effect. A variogram model can also be nested, i.e., it can be the combination of two or more component models such as nugget, spherical, or exponential. The most common nested model is the one that includes a nugget component added to one of the other models, although more complex combinations are frequently used.

The models were fitted to the variogram estimates according to an approximate weighted least squares (WLS) procedure as described in Cressie (1991, Chap. 2) (see also Pelletier and Parma (1994) for an application). According to Cressie (1991) this fitting criterion is reasonable in the sense that the more points of observations at a particular lag there are, the more weight the residual of that lag receives in the overall fit. Additionally, the lower the value of the experimental variogram, the more weight the residual receives at that lag. This means that lags closest to 0 will effectively receive more weight: this property is meaningful because it is important to obtain a good fit of the variogram especially near the origin.

A major problem in prediction is to model the measurement error and microscale variations (nugget effect) correctly. In the present study we assumed that the observed random variability at distances less than one lag was all due to microscale variation. This was supported by the fact that for every 2.5 n.miles the integrated herring density value is the mean of approximately 2×10^5 independent observations of fish density. In this case the concept of independence is not spatial independence but rather the independence in the measurement error. Thus, the influence of measurement error on a 2.5-n.mile integrated herring density value was negligible.

The utilization of the robust variogram allowed the identification of the major spatial structures of the population through the best fit model by removing most of the microscale variability. Therefore, a further analysis was undertaken to elucidate the microscale random spatial organization of the population. Various nuggets were included with the spatial structures derived from the robust variogram model.

Cross-validation

Even when a geostatistical model has been fitted on the basis of experimental variogram values, the stage of structure recognition has not yet been finished (Delhomme 1978). The adopted variogram model and kriging parameters should be validated prior to any production run (Deutsch and Journel 1992). The cross-validation method has been utilized for comparing several possible structural models (Delhomme 1978) and for optimal choices of variogram and kriging neighborhood (Simard et al. 1992; Petitgas and Poulard 1989). The kriging neighborhood is a defined area, in terms of shape and size. Only samples from this area are used in the computation of the local estimates using the kriging technique.

The cross-validation method consists of deleting one datum and using the remaining data to predict the deleted density value using the chosen variogram model and kriging parameters. Then the prediction error at each location is defined as the difference between the estimated density (or predicted, Z^*) and the measured density value (Z) (Isaaks and Srivastava 1989), $Z^* - Z$. One criterion is to maximize the correlation coefficient between the estimated and the true values, which should be close to 1 (Simard et al. 1992). However, with a substantial part of the variance included in the nugget the slope of the regression may not be unity and thus the correlation coefficient may not be useful. The mean (or expected value) of the prediction error distribution is commonly called bias and a reasonable goal for any estimation method is to produce unbiased estimates, i.e., mean error set to 0 (Isaaks and Srivastava 1989). We would also like to produce estimated values whose distribution is similar to the distribution of the true values. Their respective standard deviations or variances can be used as a measure of spread. The mean square error (MSE) is a statistical term that incorporates both the bias and the spread of the prediction error distribution and is defined as follows (Isaaks and Srivastava 1989): MSE = variance + bias^2. The MSE has been successfully utilised as a selection criterion in other earth sciences (Delfiner 1976; Delhomme 1978; Samper Calvete and Neuman 1989). Thus, in the present analysis we also preferred to use the MSE ($(SD_E)^2$ + (mean error)^2) as a method for optimal selection of the variogram models. If the variogram model adequately described the spatial dependencies implicit in the data set, then the predicted value Z^* should be close to the true value Z and the MSE should be minimized.

Mapping herring density

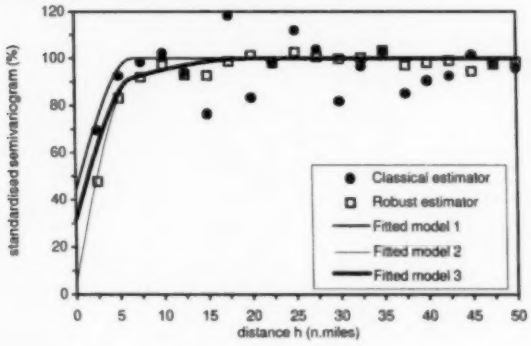
In the present study, ordinary point-kriging was used for (*i*) the variogram model fitted on the classical estimator, (*ii*) the nested variogram model applied to the robust estimator, and (*iii*) the nested

© 1996 NRC Canada

Table 1. Parameters of the fitted variogram models of herring echo abundance index on classical variogram estimator and on robust estimator of the variogram.

		Spherical 1		Spherical 2		Exponential	
	Nugget effect(c_0)	c_1	α_1 (n. miles)	c_2	α_2 (n. miles)	c_1	α_1 (n. miles)
			Classical variogram estimator				
Model 1	2590 (44%)	3307.08 (56%)	6.55	—	—		
			Robust variogram estimator				
Model 2	400 (10%)	3580 (90%)	9	—	—		
Model 3	200 (5%)					3780 (95%)	9
Model 4	200 (5%)	3148 (79%)	6.55	632 (16%)	20		

Fig. 2. Experimental standardized variograms using the classical and the robust estimator of the variogram and fitted models. Model 1 was fitted on classical estimator; model 2 was fitted on robust estimator. Model 2 gave the best fit (i.e., lower WLS criterion). Model 3 gave the lowest mean square error (MSE) in cross validation. Model parameters are presented in Table 1.



variogram model with the adjusted high nugget (minimum cross-validation MSE) applied to the classical estimator.

The model of the variogram obtained was used together with the sampling data to calculate the optimal weights attributable to each sampling unit and to estimate the density (Z^*) at an unsampled point (point-kriging):

$$Z^* = \sum_{i=1}^{N} \lambda_i Z(x_i)$$

where N is the number of samples, λ_i is the weight attributed to sample x_i, and $\Sigma\lambda_i = 1$. The N weights λ_i are calculated to ensure that the estimator is unbiased and that the estimation variance is minimal. The estimator Z^* is then said to be optimal (for a thorough presentation of the kriging theory see Journel and Huijbregts 1978, Chap. 5).

The analysis was carried out with SPSS v.6.1 (SPSS Inc.) and the following spatial analysis packages: GEO-EAS Software (Englund and Sparks 1988) was used for computing variograms and kriging and SURFER (Golden Software Inc.) for contouring.

Results

Variograms and spatial structure

The present data set was characterized by a large number of zero values, a skewed distribution with a long tail resulting from the presence of few very large values, and a rather high small-scale local variability. These are considered as characteristic properties of a typical fisheries acoustic data set.

The experimental variogram of the raw data revealed that the points were highly dispersed (Fig. 2). The variogram structure was given by the first three points of the variogram (3 × 2.5 n.miles) and showed a high nugget effect (semivariance at distance of 2.5 n. miles is high). This was due to a small number of high values scattered across the survey area, giving rise to fluctuating results. There was no indication of a trend, as there was no steady increase of the (semi-)variogram with the distance. Therefore, we assumed that the process is second-order stationary. The minimum number of pairs of data points used for the estimation of each variogram value was 108.

The omnidirectional variogram model for the raw data exhibited an autocorrelation range (a) of about 6 n.miles, which was similar to the anisotropic variogram (data not shown). The range defines the average distance beyond which points are no longer correlated spatially. The sill of the variogram quantifies the maximum level of variability between points. In our case the sill equalled the variance because the range was small compared with the dimensions of the study area (Petitgas 1993*a*). At small distances, the variogram showed a relatively high pure unresolved random structure (nugget, $c_0 = 2900$) that accounted for almost 50% of the total variance ($c_0 + c_1 = 5897.8$).

The relatively high nugget effect observed by examining the classical variogram estimator could be expected owing to the few very high values. The contribution of those values to the estimated variogram value was substantial when included at a given lag. They had a destabilizing effect that resulted in a nonrobust variogram. Both the variogram pattern and the autocorrelation range of the classical variogram estimator were not obvious. These, alongside the observed high nugget effect, made the estimation of the variogram parameters (i.e., nugget effect, range, sill) to be no more than an intelligent guess.

The experimental variogram obtained using the robust estimator of the variogram on the raw data exhibited a maximum semivariance (3980) much lower than the classical estimator owing to the reduced influence of the few very large values. For comparison purposes all of the variograms' values presented in Fig. 2 have been standardized (Rossi et al.1992) by dividing each by the sill (here, sill = variance). Except for the initial point at an average distance of 2.5 n.miles, all points were relatively less dispersed around 3980. From the various models fitted (Table 1: models 2, 3, and 4), the exponential model had a poorer fit performance than the spherical as this was evidenced by the WLS criterion. The low semivariance at the smallest distance classes on the omnidirectional variogram indicated that a small-scale structure could be nested within the large-scale one. The two-structure omnidirectional model (Fig. 2), consisting of a spherical function with a range of

© 1996 NRC Canada

Table 2. Model parameters (standardized) and cross-validation errors.

Variogram model parameters standardized (%)	Max. no of points	Mean prediction error	SD_E	MSE
c_0=5	18	−0.218	57.900	3352.46
c_1=79	16	−0.123	56.769	3222.73
c_2=16	14	−0.123	56.395	3180.41
c=100	12	−0.132	56.623	3206.18
	10	−0.120	56.708	3215.81
	8	−0.213	57.233	3275.66
c_0=12	18	−0.053	57.147	3265.78
c_1=72	16	−0.062	56.401	3181.08
c_2=16	14	−0.070	56.190	3157.32
c=100	12	−0.071	56.401	3181.08
	10	−0.066	56.405	3181.53
	8	−0.029	56.435	3184.91
c_0=19	18	−0.092	57.009	3250.03
c_1=65	16	−0.063	56.198	3158.22
c_2=16	14	−0.041	56.023	3138.58
c=100	12	−0.035	56.119	3149.34
	10	−0.033	56.173	3155.41
	8	−0.020	56.237	3162.60
c_0=25	18	−0.106	57.310	3284.45
c_1=59	16	−0.012	56.128	3150.35
c_2=16	14	−0.005	55.929	3128.05
c=100	12	−0.008	56.033	3139.70
	10	−0.006	56.068	3143.62
	8	−0.023	56.168	3154.84
c_0=32*	18	0.008	56.897	3237.27
c_1=52*	16	0.017	56.001	3136.11
c_2=16*	14	0.012	55.860	3120.34*
c=100	12	−0.001	55.933	3128.50
	10	0.011	55.998	3135.78
	8	−0.022	56.031	3139.47
c_0=38	18	0.060	56.047	3141.27
c_1=46	16	0.030	56.020	3138.24
c_2=16	14	0.029	55.882	3122.80
c=100	12	0.038	56.033	3139.70
	10	0.043	56.060	3142.73
	8	−0.042	56.215	3160.13
c_0=42	18	0.021	56.983	3247.06
c_1=42	16	−0.018	56.099	3147.10
c_2=16	14	−0.059	56.021	3138.36
c=100	12	−0.095	56.157	3153.62
	10	−0.116	56.315	3171.39
	8	−0.079	56.352	3175.55
c_0=50	18	0.211	57.264	3279.21
c_1=34	16	0.103	56.452	3186.84
c_2=16	14	0.080	56.372	3177.81
c=100	12	0.094	56.325	3172.51
	10	0.103	56.403	3181.31
	8	−0.110	56.387	3179.51

Note: $c_0 + c_1 + c_2 = c$ = constant for all models. Proportions of nugget c_0 and first component c_1 are varied (c_0 from 5 to 50%). Mean prediction error is the estimation error (estimated – observed). SD_E is the standard deviation of the errors of the estimates from the observed values obtained using the variogram model and the kriging neighbourhood at the sample points. MSE = $(SD_E)^2$ + (mean prediction error)^2. The minimum MSE model ($\gamma(\mathbf{h})$ = nugget (32%) + spherical (sill = 52%, range = 6.55 n.miles) + spherical (sill = 16%, range = 20 n.miles), c = 5897, shown with asterisks) is used for computing the map in Fig. 6.

about 6–7 n.miles plus a second spherical function with a range of 20 n.miles both superimposed over a low nugget effect performed significantly better (it gave the best fits) than the single spherical model, and it was thus used for the kriging estimation. The nugget effect c_0 represented a maximum of about 5% of the overall variability. The first structure (c_1 = 3150, a_1 = 6.55 n.miles) accounted for 79% of the overall variability whereas the second one (c_2 = 630, a_2 = 20 n.miles) accounted for the remaining 16%.

The utilisation of the robust estimator of the variogram effectively helped to reveal nested structures and adjust autocorrelation ranges (J. Rivoirard, Centre de Géostatistique, École Nationale Supérieure de Paris, Fontainbleau, personal communication) by putting adjusted weight on the few very high values and by reducing the contribution of the microscale variability. The resulting variogram was well behaved but contained no component to express the variability introduced by these high values. Therefore, the nugget calculated from the robust estimator variogram was underestimated. We believe that the small number of high-value observations are authentic and characteristic of the particular stock in the sense that they are repeatedly observed, although in different locations over a number of years of surveys. A variogram that does not take them into account is only partially describing the underlying real structure of the stock. A model on the classical estimator of the variogram is the only one that will enable us to reproduce the data total variance. On the basis of the above observations a further analysis was undertaken to determine and hence to incorporate the most appropriate nugget in the variogram model.

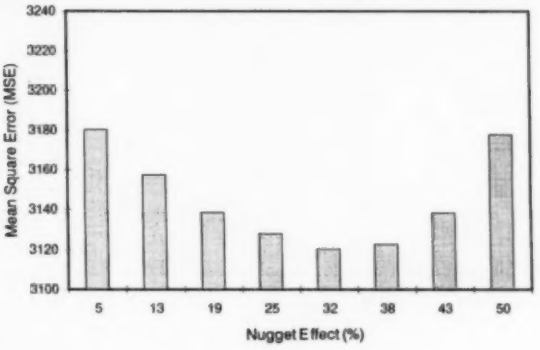
Cross-validation

Cross-validation tests were performed retaining the structures revealed by the best fit model (i.e., nested with two spherical functions and a_1 = 6.55, a_2 = 20 n.miles) and changing the proportion of the unresolved small-scale variability to the short-range structural component. We then assessed various structures using the MSE as the locally optimal selection criterion. For each structure tested, the minimum MSE occurred when 14 points were used in the cross-validation (Table 2). This supported the idea that taking the spatial structure into account is advantageous when computing the local estimates. The standardizd residuals were found to have a mean close to 0 and a SD close to 1 (global unbiasness condition). The slopes of the regressions of the true values against the estimates were not 1, mainly because of the high nugget. This made the correlation coefficient of the regression inappropriate for use as a selection criterion.

Cross-validation revealed that a nugget of about 32% (c_0 = 1250, Fig. 3) of the total variance was the more effective in terms of MSE (values with asterisks in Table 2; Fig. 3). This corresponded to an omnidirectional variogram modelled by a two-structure isotropic model consisting of two spherical functions plus a nugget effect (model 3 in Fig. 2). A meso-scale structure of an approximate range of 6.5 n. miles, which represented about 52% of the total variability, appeared to be nested in the larger 20-n.mile range structure representing the remaining 16% of the variability. The percentages of the three different levels of variability (micro-, meso-, and large-scale) were retained, and combined with the overall variance of the raw data (5897.8) they were used for the kriging estimation.

© 1996 NRC Canada

Fig. 3. Cross-validation MSE derived for different values of nugget, $c_0 + c_1 = 3350$ (constant). Model parameters are presented in Table 2.



In conclusion, the robust variogram estimator enabled the underlying spatial dependence to be revealed (i.e., larger scale nested structure) and identified the correlation ranges. Cross-validation MSE correctly tuned the nugget effect.

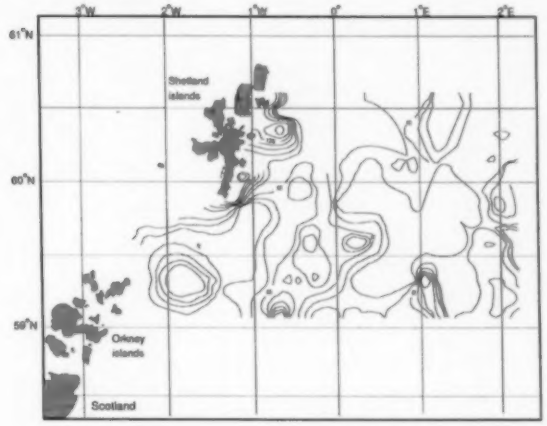
Mapping

Kriging was carried out on a grid of approximate 2-n.mile spacing. The use of 14 sample points in the kriging neighbourhood enabled the in-between transect estimates to approximate the local zone mean (i.e., data neighbourhood average). This is important because the unsurveyed area values were unknown. The observed autocorrelation ranges and intertransect distances were also considered in the choice of the kriging neighbourhood. In general, the use of more samples in a weighted linear combination increases the smoothness of the estimates. This is why good estimation inevitably leads to smoothing. As a result there are fewer extremely low and very high estimates than there are extremely low or very high observations.

Three contour maps generated using three different variogram models are presented in Figs. 4, 5, and 6. As would be expected, all three maps showed the same general features. There was an area of high fish concentration along the east coast of the Shetland Islands. A generally low density was seen in the middle of the study area (rectangle bounded by 59°30′ and 60°30′N and by 00°00′ and 02°00′E). Higher densities were also apparent south of 59°45′N. Maps revealed that herring tended to gather both in meso-scale discrete patches and in larger scale aggregations. The meso-scale patches represented herring schools; this was also confirmed by cross-examining the echograms and the integrator cumulative output.

The map generated using the variogram derived from the raw data (Fig. 4) was very smooth. This was the result of the relatively high level of unresolved spatial structure (a high nugget effect). It showed relatively little structure away from the areas of high density. Figure 5, generated from the nested variogram model, with a small nugget effect (5% of the total variance), appeared to show much more detail. Concentric rings indicated that many individual values were influencing the plot. However, in this case, because of the very low nugget, the map was largely derived directly from the observations and was dominated by the high point values. In general, the higher the nugget effect, the smoother the map. The third map (Fig. 6)

Fig. 4. Contour plot of kriged grid derived from model 1 fitted on the classical estimator of the variogram (Table 1: model 1; $c_0 = 45\%$, $c_1 = 55\%$).



was derived from the variogram calculated to give the best cross-validation output with a nugget of about 32% of the total variance. On this map it was possible to see regions of high and low fish density, and the structures were on a scale that should allow comparison with ancillary data.

Discussion

The main aim of the present study was to develop the most appropriate methodology for analysing the structure and mapping the stock distribution of herring derived from an acoustic survey east of the Shetland Islands in 1992. The key to a successful estimation is to choose a variogram that captures the pattern of spatial continuity of the data set (Isaaks and Srivastava 1989). In the present study, the distribution of the data was highly skewed, with a small number of very high values, but representative observations and a large number of zero values; these features are generally encountered in acoustic surveys. The presence of the few very high values had a destabilizing effect that masked both the variogram pattern and the autocorrelation ranges and gave rise to a high nugget effect. The robust estimator of the variogram revealed the underlying spatial dependence (larger scale structural component) and helped to better define the correlation ranges. The correct proportion of the nugget effect was accurately estimated using the minimum cross-validation MSE as the optimal criterion. The procedure ensured that the optimal map of the stock distribution was obtained from the available data set, thus allowing the biologist to compare it with other ancillary data (temperature, salinity, etc.).

It was then possible to study the population structure and any postulated relationships with the environment. The maps clearly showed high-density patches in some parts of the area and a general absence of herring in the central part. Of the three maps produced, only the third represented the sort of distribution that an experienced biologist might expect, with a meaningful, or at least understandable, interpolation between the tracks. The first map conveyed little information because it was very smoothed, whereas the second appeared to be largely

© 1996 NRC Canada

Fig. 5. Contour plot of kriged grid derived from variogram model fitted on the robust variogram estimator (Table 1: model 4; $c_0 = 5\%$, $c_1 = 79\%$, $c_2 = 16\%$).

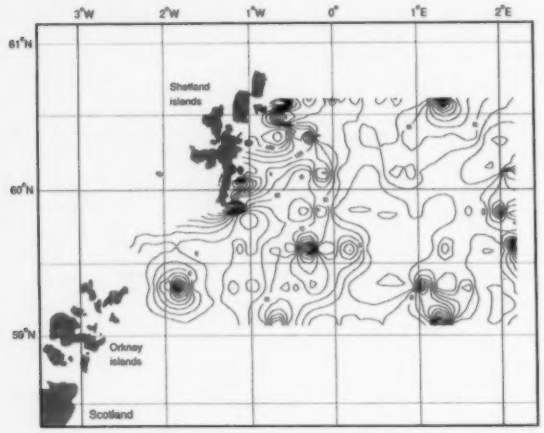
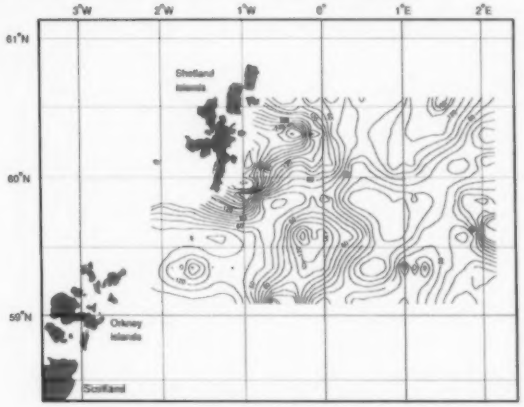


Fig. 6. Contour plot of kriged grid derived using the variogram model g(**h**) = nugget (32%) + spherical (sill = 52%, range = 6.55 n.miles) + spherical (sill = 16%, range = 20 n.miles), fitted on the classical estimator (Table 2, asterisks; Fig. 2, model 3).



driven by the variation seen along the track. This choice between the maps is, by nature, subjective; however, it is important to note that the parameters calculated in the analysis allowed the production of a map that conveys the maximum amount of information to the biologist. The structure and location of the patches observed in the maps imply that the formation of such patches depends on a number of factors. Two different ranges of structures were identified in the variogram. The first had a range of about 6.5 n.miles and accounted for 52% of the overall variability. Unpublished work by D.G. Reid[2] would suggest that it is largely related to the topography and substrate of the seabed. Oceanographic factors such as water temperature and salinity (Maravelias and Reid[3]) and also water depth (Reid et al.1993) are likely to affect spatial structure and habitat utilization, resulting in spatial segregation. This may explain the longer range structure, about 20 n.miles, which accounted for 16% of the overall variability. Hence, the inclusion of the second nested spherical function in the model (which was revealed from the use of the robust variogram estimator) was also supported by the fact that there was qualitative information about the phenomenon that explains it (Isaaks and Srivastava 1989). Maravelias and Reid (1995*a*) proposed that this is best seen in terms of the hydrography of the area and the presence of fronts and currents as well as the transition from mixed to stratified waters. Higher productivity areas, associated with mixing owing to currents and frontal systems, may stimulate herring to aggregate in response to the greater availability of food.

Although significant improvements in variogram estimation and mapping were obtained, there is no pretence that applying this methodology is the only or necessarily the best approach to robust estimation of the variogram (Cressie and Hawkins 1980) and to optimal mapping of the population in the presence of high local variability. Alternative techniques have been developed to deal with similar problems (i.e., disjunctive kriging and conditional expectation). The application of the present method is one simple way to accomplish a robust analysis and optimal mapping using ordinary kriging when fish distributions are highly aggregated, such that a few high-value observations can strongly influence the estimation in an acoustic survey. Rivoirard (1990) suggested that when our aim is to estimate the variable itself, $Z(x)$, linear kriging is more robust in practice (compared with disjunctive or indicator kriging) because it requires less stringent hypotheses. In the method presented here, which aimed to analyse the actual density structure of the whole population (local estimation), particular emphasis was given to the large densities because these were considered essential in the regionalization of the fish population. Conversely, the purpose of disjunctive kriging is to analyse how the different ranges of values (the different quantiles of the distribution, i.e., high, medium, low) are related to each other in space (Petitgas 1993*b*). Further, indicator kriging produces estimates of the conditional probability distribution for particular cutoffs (Isaaks and Srivastava 1989; Maravelias and Reid 1995*b*).

Finally, it should be pointed out that a variogram is a descriptive statistical model for the particular realization of the phenomenon under study. As conceptualizations of the phenomenon that allow us to predict what is happening at locations where we do not have samples, models are neither right nor wrong; they can only be judged as appropriate or inappropriate (Isaaks and Srivastava 1989). A model is never unique and can be judged only on its adequacy to solve the particular problem at hand (Journel 1986). If one model performs better than another on a particular data set, it does not mean, in general, that the model will always perform better (Davis 1987). In this context, Matheron (1989) criticized the fit of a model on the experimental variogram with scepticism: even when a model fits the experimental points well, it has to be assumed

[2] D.G. Reid. Relationships between herring school distribution and sea bed substrate derived from RoxAnn. Presented at the ICES International Symposium on Fisheries and Plankton Acoustics, Aberdeen, Scotland, 12–16 June 1995.

[3] C.D. Maravelias and D.G. Reid. Structural analysis of fisheries acoustic data in relation to environmental factors: an application of geostatistical tools. Presented at the ICES International Symposium on Fisheries and Plankton Acoustics, Aberdeen, Scotland, 12–16 June 1995.

© 1996 NRC Canada

that it also fits the nonsampled points (radical error). In other words, if a particular model fits the present data set, this does not necessarily imply that the model also describes adequately the underlying structure of the population. Furthermore, stationarity is a constitutive property of the random function model, not an intrinsic property of the phenomenon under study. Care should be taken when attempting linkage between the well-defined stationarity property (of a model) and the subjective notion of homogeneity (of a population) (Journel 1986). Hence, the different computational steps of the analysis should not be applied without always bearing in mind the assumptions made or without having a good understanding of the ecology of the studied species. Additional environmental variables collected alongside the acoustic data can also be used. These may explain part of the observed spatial variation and help to elucidate the spatial distribution of species in relation to their environment. They may also prove useful in defining areas that require intense sampling effort owing to high variability.

In conclusion, the present study illustrates a new approach to the fitting of variogram models to highly skewed acoustic survey data, where the few, characteristic high values cannot be discarded as outliers. The combination of determining model parameters from the robust estimator variogram and iterating to an appropriate nugget using cross-validation techniques allows much more intuitively realistic maps to be generated.

Acknowledgments

This study would not have been possible without the financial support of a European Community research fellowship (DG XIV, Fisheries Sector) awarded to C.D.M. We thank J. Rivoirard, Centre de géostatistique, École nationale supérieure des mines de Paris, Fontainebleau, for useful comments on an earlier version of this paper. We also thank G. Swartzmann and D. Pelletier for critical remarks on the initial manuscript.

References

Conan, G.Y., Maynou, F., Stolyarenko, D., and Mayer, L. 1994. Mapping and assessment of fisheries resources with coastal and depth constraints, the case study of snow crab in the Bay of Islands Fjord (Newfoundland). ICES C.M. 1994/B+D+G+H: 4. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Cressie, N.A.C. 1991. Statistics for spatial data. John Wiley and Sons, New York.

Cressie, N.A.C., and Hawkins, D.M. 1980. Robust estimation of the variogram, I. J. Int. Assoc. Math. Geol. **12**: 115–125.

Davis, B.M. 1987. Uses and abuses of cross-validation in geostatistics. Math. Geol. **19**: 241–248.

Delfiner, P. 1976. Linear estimation of non-stationary spatial phenomena. *In* Advanced geostatistics in the mining industry. *Edited by* M. Guarascio, M. David, and C. Huijbregts. D. Reidel Publishing Co., Dordrecht, The Netherlands. pp. 49–68.

Delhomme, J.P. 1978. Kriging in the hydrosciences. Adv. Water Resour. **1**: 251–266.

Deutsch, C.V., and Journel, A.G. 1992. GSLIB geostatistical software library and user's guide. Oxford University Press, New York.

Englund, E., and Sparks, A. 1988. GEO-EAS (geostatistical environmental assessment software) user's guide. U.S. Environmental Protection Agency, Las Vegas, Nev.

Foote, K.G. 1983. Linearity of fisheries acoustics: with additional theorems. J. Acoust. Soc. Am. **73**: 1932–1940.

Krige, D.G., and Magri, E.J. 1982. Studies on the effects of outliers and data transformation on variogram estimates for a base metal and a gold ore body. Math. Geol. **14**: 557–564.

Journel, A.G. 1986. Geostatistics: models and tools for the earth sciences. Math. Geol. **18**: 119–140.

Journel, A.G., and Huijbregts, C.J. 1978. Mining geostatistics. Academic Press, London.

Isaaks, E.H., and Srivastava, R.M. 1989. Applied geostatistics. Oxford University Press, New York.

Maravelias, C.D., and Reid, D.G. 1995*a*. Relationship between herring (*Clupea harengus*, L.) distribution and salinity and temperature in the northern North Sea. Sci. Mar. **59**(3–4): 427–438.

Maravelias, C.D., and Reid, D.G. 1995*b*. Spatial relationships between herring, plankton and oceanographic conditions in the North Sea (ICES Div. IVa). ICES C.M. 1995/Q:16. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Matheron, G. 1963. Principles of geostatistics. Econ. Geol. **58**: 1246–1266.

Matheron, G. 1971. The theory of regionalized variables and its applications. *In* Cahiers du CMM. No. 5. École nationale supérieure des mines de Paris, Fontainebleau, France.

Matheron, G. 1989. Estimating and choosing: an essay on probability in practice. Springer-Verlag, Berlin, Germany.

Pelletier, D., and Parma, A.M. 1994. Spatial distribution of Pacific halibut (*Hippoglossus stenolepis*): an application of geostatistics to longline survey data. Can. J. Fish. Aquat. Sci. **51**: 1506–1518.

Petitgas, P. 1993*a*. Geostatistics for fish stock assessments: a review and an acoustic application. ICES J. Mar. Sci. **50**: 285–298.

Petitgas, P. 1993*b*. Use of a disjunctive kriging to model areas of high fish density in pelagic acoustic fisheries surveys. Aquat. Living Resour. **6**: 201–209.

Petitgas, P., and Poulard, J.C. 1989. Applying stationary geostatistics to fisheries: a study on hake in the Bay of Biscay. ICES C.M./G:62. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Reid, D.G., Williams, D., Gambang, A., and Simmonds, E.J. 1993. Distribution of North Sea herring and their relationship to the environment. ICES C.M./H:23. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Rivoirard, J. 1990. Introduction to disjunctive kriging and nonlinear geostatistics. *In* Course C–143. Centre de géostatistique, École nationale supérieure des mines de Paris, Fontainebleau, France.

Rossi, R.E., Mulla, D.J., Journel, A.G., and Franz, E.H. 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. Ecol. Monogr. **62**: 277–314.

Samper Calvette, F.J., and Neuman, S.P. 1989. Geostatistical analysis of groundwater quality data from the Madrid basin using adjoint state maximum likelihood cross-validation. *In* Geostatistics. Vol. 2.M. *Edited by* M. Armstrong. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 725–736.

Simard, Y., Legendre, P., Lavoie, G., and Marcotte, D. 1992. Mapping, estimating biomass, and optimizing sampling programs for spatially autocorrelated data: case study of the northern shrimp (*Pandalus borealis*). Can. J. Fish. Aquat. Sci. **49**: 32–45.

Simard, Y., Marcotte, D., and Bourgault, G. 1993. Exploration of geostatistical methods for mapping and estimating acoustic biomass of pelagic fish in the Gulf of St. Lawrence: size of echo-integration unit and auxiliary environmental variables. Aquat. Living Resour. **6**: 185–199.

Simmonds, E.J., Dommasnes, A., Aglen, A., Corten, A., and Reid, D.G. 1993. 1992 ICES co-ordinated acoustics survey of ICES divisions IVa, IVb, and VIa. ICES C.M./H:11. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Sullivan, P.J. 1991. Stock abundance estimation using depth-dependent trends and spatially correlated variation. Can. J. Fish. Aquat. Sci. **48**: 1691–1703.

© 1996 NRC Canada

Comparison of behavioural and morphological measures of visual resolution during ontogeny of roach (*Rutilus rutilus*) and yellow perch (*Perca flavescens*)

Josef Wanzenböck, Monika Zaunreiter, Christina M. Wahl, and David L.G. Noakes

Abstract: Changes in visual resolution of two fish species, the yellow perch, *Perca flavescens*, and the roach, *Rutilus rutilus*, were measured during ontogenetic development from first feeding to adult fish using (*i*) morphological measurements (eye lens diameter and cone density) and (*ii*) a behavioural correlate of visual resolution (reactive distance). Both methods were used to calculate visual resolution expressed as minimum separable angle (MSA). Morphological estimates of MSA were similar in both species whereas behaviourally determined MSA values were higher in roach. Initially, predicted performance based on morphological measurements was 3–10 times better than behavioural measurements of MSA for both species. With growth, behavioural measures of visual resolution improved, resulting in similar MSA values at 35 mm standard length in perch and 100 mm standard length in roach. Above these sizes, both species outperformed the morphologically predicted MSA values. The sensitivity of calculations of MSA from eye lens diameter and cone density to both variables was analysed and ways to correct for differences in methods (e.g., the use of fixed or unfixed material, whether cell counts were made within specific retinal areas, and changes in Mathiessen's ratio) were exemplified to facilitate comparison of absolute MSA values.

Résumé : Les changements survenus dans la résolution visuelle de deux espèces de poissons, la perchaude, *Perca flavescens*, et le gardon, *Rutilus rutilus*, ont été mesurés pendant l'ontogenèse, entre le début de l'alimentation et l'âge adulte, par les moyens suivants : (*i*) des mesures morphologiques (diamètre du cristallin et densité des cônes) et (*ii*) un corrélat comportemental de la résolution visuelle (distance de réaction). Les deux méthodes ont été employées pour calculer la résolution visuelle exprimée sous forme d'angle minimum de séparation (AMS). Les estimations morphologiques de l'AMS étaient similaires chez les deux espèces tandis que leurs valeurs déterminées par le comportement étaient plus élevées chez le gardon. Au départ, la performance prédite en fonction des mesures morphologiques était 3–10 fois meilleure que les mesures comportementales de l'AMS pour les deux espèces. Avec la croissance, les mesures comportementales de la résolution visuelle se sont améliorées, de sorte que les valeurs de l'AMS étaient similaires à 35 mm de longueur standard chez la perchaude et 100 mm de longueur standard chez le gardon. Au-delà de ces tailles, les deux espèces dépassaient les valeurs de l'AMS prédites par les mesures morphologiques. La sensibilité aux deux variables des calculs de l'AMS à partir du diamètre du cristallin et de la densité des cônes a été analysée, et nous donnons des exemples des façons de corriger les différences dans les méthodes (par example, l'emploi de matériel fixé ou non fixé, le fait que les dénombrements cellulaires étaient faits dans certaines zones spécifiques de la rétine, et les changements dans le rapport de Mathiessen) afin de faciliter la comparaison des valeurs absolues de l'AMS.
[Traduit par la Rédaction]

Introduction

Vision is the primary sense most fishes use in searching for food (reviewed by Lazarro 1987; O'Brien 1987), and there is an extensive literature on the subject (for reviews see Munz and McFarland 1978; Lythgoe 1979; Douglas and Djamgoz 1990; McFarland 1992). Early studies on the visual abilities of fish were primarily morphological and physiological. These included detailed investigations of the optical properties of eye parts, microscopic anatomy (e.g., Tamura 1957; Northmore and Dvorak 1979), as well as performance estimates using the optokinetic response (Rahmann et al. 1979; Neave 1984; Pankhurst et al. 1993). With the increasing emphasis on behavioural aspects of fish–zooplankton interactions and their importance in foraging models, behavioural measures of visual resolution have become important.

Commonly, the distance at which the predator first reacts to a prey item (reactive distance (RD)) is used to estimate the behavioural visual ability of the predator. The RD is an important factor in foraging models used to calculate encounter rates with prey and subsequently assess many aspects of fish–

Received April 20, 1995. Accepted February 15, 1996.
J12882

J. Wanzenböck. Institute of Limnology, Austrian Academy of Sciences, Gaisberg 116, A-5310 Mondsee, Austria.
M. Zaunreiter. Institute for Zoology, University of Salzburg, Hellbrunnerstraßβe 34, A-5020 Salzburg, Austria.
C.M. Wahl. Department of Anatomy, T5015 VRT, Cornell College of Veterinary Anatomy, Cornell University, Ithaca, NY 14853, U.S.A.
D.L.G. Noakes. Institute of Ichthyology, University of Guelph, Guelph, ON N1G 2W1, Canada.

© 1996 NRC Canada

Table 1. Dimensions of experimental tanks, water volumes, and water depth used with fish (both species) of different sizes.

Fish		Experimental tanks		
Age (days)	SL (mm)	Diameter (cm)	Volume (mL)	Water depth (cm)
12–26	7–13	15	150	0.85
26–40	13–18	20	300	0.95
40–48	18–20	25	1 000	2.04
48–60	20–25	25	2 000	4.08
60–90	25–50	30	3 000	4.24
90–912	50–95	100	40 000	5.09

zooplankton interactions such as prey selectivity, habitat choice, or top-down effects on the whole community (reviewed by Northcote 1988; Carpenter and Kitchell 1993).

The literature on visual resolution estimated from retinal morphology and eye dimensions as well as estimates from optokinetic response studies are of limited use for ecological purposes, since information is lacking on how these estimates are scaled to actual performance, i.e., prey detection ability in foraging fish. This scaling changes during certain phases of ontogenetic development (Hairston et al. 1982; Neave 1984; Li et al. 1985; Walton et al. 1992; Miller et al. 1993) but there are only a few comprehensive studies detailing the relationships between morphological and physiological estimates and actual prey detection abilities over the entire ontogenetic development from first feeding larva to adult fish (Walton et al. 1994). Such changes in scaling are expected as developmental refinements facilitate translation from the morphological basis to behavioural performance. These include, in part, changes in myopia (Pankhurst et al. 1993), development of cone patterns (Browman et al. 1990), convergence of cones onto ganglion cells (Zaunreiter et al. 1991), development of visual centres in the brain (Brandstätter and Kotrschal 1989), and learning mechanisms (Noakes and Godin 1988).

The present study describes changes in the morphological and behavioural components of the visual system throughout ontogeny in roach (*Rutilus rutilus*) and yellow perch (*Perca flavescens*). Two well-defined estimates of visual resolution that are based on these components are compared for these animals. Morphological calculations of visual resolution are based on cone density and cone topography as well as eye and lens diameters. Behavioural data were gathered using RD experiments. The following comparison between morphological and behavioural estimates of visual resolution is important for placing published data in an ecological context: how does morphological development translate to behavioural performance, and ultimately to predator–prey interactions? The goal is to better use morphologically determined minimum separable angle (MSA) values from the literature and predict behavioural performance at least as an approximation in case behaviourally determined MSA values are not available.

Materials and methods

Behavioural studies

Fish used in the behavioural experiments were raised from fertilized eggs in the laboratory. Groups of three individual fish were placed in round experimental vessels that had diameters at least 10 times the standard length (SL) of the fish (Table 1). The cylindrical side walls of the experimental vessels were made of white plastic and the glass bottom was covered by blue graph paper (1-mm grid). Illumination was provided by one overhead (60-cm distance) halogen lamp (150 W), resulting in a light intensity of 12 000 lx at the water surface. Prey for the experiments came from laboratory cultures and distinct length classes were produced by sequential sieving. Prey items consisted of brine shrimp nauplii (prey length: 0.4 ± 0.06 mm (mean ± SD, $n = 100$), used with *P. flavescens*), *Ceriodaphnia* sp. (prey length: 0.49 ± 0.05 mm ($n = 100$) and 0.65 ± 0.1 mm ($n = 100$), used with both fish species), and *Daphnia magna* (prey length: 1 ± 0.14 mm ($n = 100$) and 2 ± 0.18 mm ($n = 100$), used with *R. rutilus* >60 mm SL). Single prey items were introduced to the experimental vessels and captures by any of the three fish were recorded on videotape. A maximum of 30 prey items was used in any one experiment to prevent satiation effects.

Experiments were videotaped either using a black and white video camera and a U-matic video system for roach (for more technical details see Wanzenböck and Schiemer 1989) or a colour video camera and an S-VHS tape system for yellow perch. Analysis of videotapes consisted of determining the exact point of reactions to prey items as seen by sudden changes in swimming direction and (or) acceleration. At the moment of reaction the distance between prey and the snout of the fish (the RD) was measured. Only those attacks in which prey was more than 50 mm from the side walls and in which reactions were preceded by a period of search were included in the analysis. RD and prey size (h) were used to calculate the MSA, which is given by MSA = 2(arctan(0.5 h/RD)). Smaller MSA values correspond to better visual resolution. Theoretically, MSA values should be similar for all prey sizes at a certain fish size in these experiments. Data for yellow perch are original whereas those for roach are taken from Wanzenböck and Schiemer (1989) and from Grabner (1990).

Morphological studies

Rutilus rutilus

Fish were caught by gill netting in a backwater of the Danube River near Vienna, Austria. After anaesthetization with MS-222 they were perfused with buffered 10% formaldehyde solution. Eyes were stored in fixative for at least 2 weeks before they were rinsed in phosphate buffer and retinae were dissected out of the eyeballs. Depending on fish size, 8–16 pieces of retina from different areas of the eye were dehydrated in ethanol and embedded in historesin. Tangential sections were cut to 3 µm, mounted on glass slides, and stained with haematoxylin and eosin.

Cones were counted by means of light microscopy on each of the pieces of retina in three different, randomly selected positions. Each counting position measured 250, 640, or 1220 µm^2 (for fish sizes SL < 100, 100 < SL < 200, and SL > 200 mm, respectively). Mean values of the three counts were calculated for each of the 8–16 pieces from all over the retina. In this analysis only mean values of these pieces from different retinal areas are used. For more details, especially cone cell density differences over the entire retina, see Zaunreiter et al. (1991), from which the data on cone density and on diameters of fixed lenses are taken.

Because the amount of shrinkage of the eye and the lenses in different ontogenetic stages was not exactly known, additional measurements of eye and lens diameters were made on fresh eyes dissected immediately after killing fish with heavy doses of MS-222. These represent previously unpublished data.

Perca flavescens

Eye and lens diameters were measured on fresh material. Retinas were dissected from fresh eyes, separated from the pigment epithelium, and floated, photoreceptor side up, onto glass slides. Cone density was determined using light microscopy with Nomarski differential interference contrast optics. The number of cones per 0.1 mm^2 was measured on 6–12 pieces from the central posterior part of the retina. This

© 1996 NRC Canada

Fig. 1. (*a*) Reactive distance of *P. flavescens* versus standard length for two prey sizes: 0.4 mm, solid symbols; 0.49 mm, open symbols. (*b*) Minimum separable angle (MSA) calculated from data in *a* versus standard length for *P. flavescens*. Regression equation: $\log y = 3.5 - 1.2 \log x$, $r^2 = 0.76$, $n = 13$. (*c*) MSA values for *R. rutilus*. Prey sizes: 0.49 mm, circles; 0.65 mm, triangles; 1 mm, inversed triangles; 2 mm, squares. Regression equation: $\log y = 3.8 - 1.2 \log x$, $r^2 = 0.96$, $n = 23$. In general, symbols represent mean values, and error bars represent standard deviations.

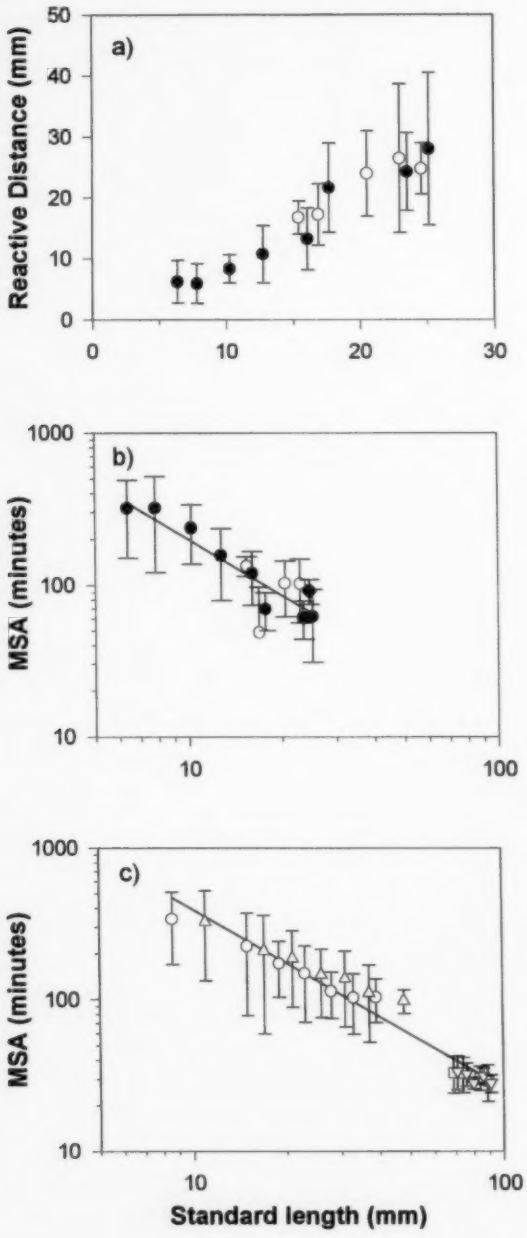
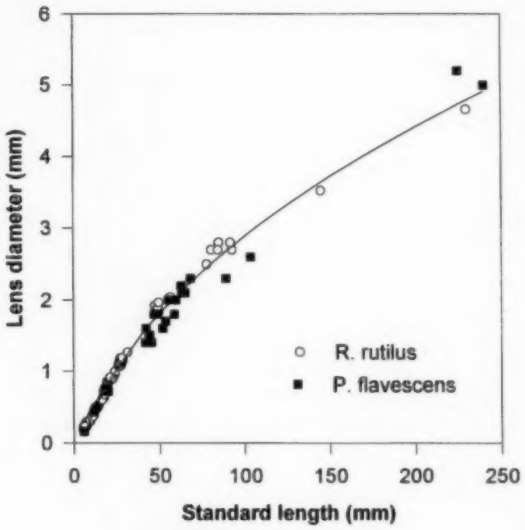


Fig. 2. Lens diameter versus standard length of *R. rutilus* and *P. flavescens*. Regression equation: $y = -0.802 + 0.369x^{1/2}$, $r^2 = 0.97$, $n = 103$.



corresponds to the area of the forward visual field and cone density is known to be highest there (Hairston et al. 1982; Browman et al. 1990). For more details on the morphological processing of this species' eyes see Wahl et al. (1993), from which all morphological data on yellow perch are taken.

Results

Behavioural study

The RD towards each prey size increased linearly with length of *P. flavescens* (Fig. 1*a*). Consequently, MSA as calculated from RD and prey size changed exponentially with SL (Fig. 1*b*). As expected theoretically, differences in MSA values related to different prey sizes were insignificant (analysis of covariance (ANCOVA) of log-transformed data with fish size being a covariable, $p > 0.1$). MSA data for different prey sizes were pooled to calculate one regression line for the relationship between MSA and fish SL. Figure 1*c* shows the corresponding data set for *R. rutilus*, taken from Wanzenböck and Schiemer (1989) and from Grabner (1990). In this species too, changes in MSA with fish size can be described by one linear model (when data are log transformed) for all prey sizes used. However, there were interspecific differences in MSA values (ANCOVA of log-transformed data, $p < 0.01$). At any given fish size *P. flavescens* had a lower MSA value and therefore better visual resolution than *R. rutilus*. Nevertheless, the improvement of visual abilities was similar in both species as seen by similar slopes of the regression lines.

Morphological studies

The relationship between lens diameter (fresh lenses) and fish SL was similar for the two species (ANCOVA of untransformed data, $p > 0.1$). Therefore, one curvilinear equation could be fitted to these data, indicating that lens growth slows as fish grow (Fig. 2). To estimate possible effects of lens

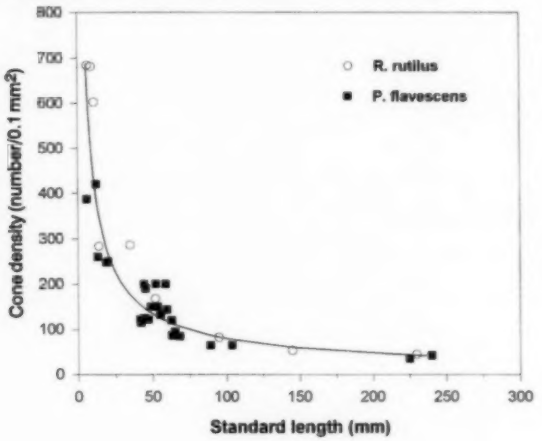
© 1996 NRC Canada

Table 2. Variables of eye morphology for a range of sizes of *Rutilus rutilus*.

SL	ED	LD	Mathiessen ratio	LD fresh	Ratio 1	Cd avg.	Cd a.t.	Ratio 2
6.5	0.4	0.05	8.0	0.22	4.4	683	722	1.06
9.0	0.5	0.10	5.0	0.33	3.3	681	681	1.00
11.0	1.0	0.30	3.3	0.42	1.4	602	617	1.03
14.0	1.5	0.40	3.8	0.55	1.4	283	323	1.14
35.0	3.0	1.00	3.0	1.33	1.3	286	288	1.01
52.0	3.8	1.30	2.9	1.84	1.4	167	219	1.31
95.0	6.8	2.50	2.7	2.78	1.1	82	101	1.24
145.0	8.5	3.00	2.8	3.52	1.2	53	58	1.11
230.0	12	4.00	3.0	5.14	1.3	44	48	1.09
Average			3.83		1.86			1.11

Note: SL, standard length (mm); ED, eye diameter (mm, fixed eyes); LD, lens diameter (mm, fixed); Mathiessen ratio, ED/LD (both fixed); LD fresh, lens diameter of fresh lenses (mm); ratio1, LD fresh/LD; CD avg., cone density (average values for the whole retina, number per 0.1 mm^2); CD a.t., cone density in area temporalis; ratio 2, CD a.t./CD avg.

Fig. 3. Cone density of *R. rutilus* and *P. flavescens* versus standard length. Regression equation: $y = 2597x^{-0.7356}$, $r^2 = 0.89$, $n = 34$.



shrinkage we compared measurements of fresh and fixed lenses for different sizes of *R. rutilus* (Table 2). In small fish, lenses shrank considerably more (see ratio 1 in Table 2) than in larger fish, indicating higher water content in smaller lenses.

Cone density counts by different investigators using different methods and two different species were surprisingly similar (Fig. 3). No interspecific differences could be detected (ANCOVA of log-transformed data, $p > 0.1$). A general relationship between cone density and SL for both species was found to be represented by a negative exponential equation (Fig. 3). To account for differences in method (i.e., average of counts from all over the retina for *R. rutilus* and counts from the area temporalis in *P. flavescens*) we compared such differences in cone topography for *R. rutilus* (Table 2). On average, cone density was 11% (average ratio 2 in Table 2) higher in the area temporalis than the average values from the entire retina.

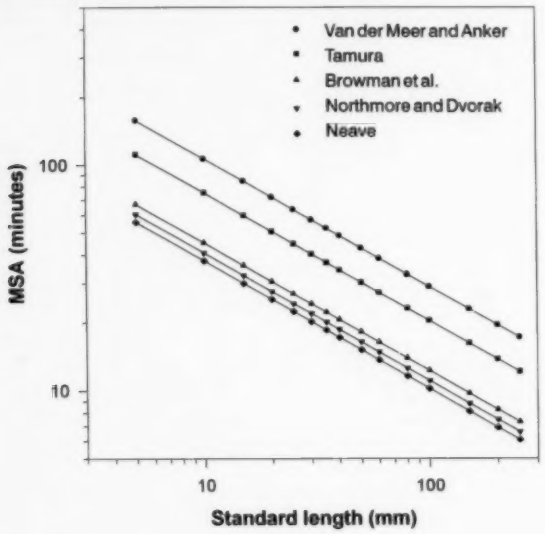
Using the regressions for lens diameter and cone density versus SL, we calculated MSA values for a range of fish sizes (5–250 mm) using different formulae given in the literature. Negative exponential functions were fitted to the data and plotted in Fig. 4. A wide range of MSA values was obtained, depending on which formula was used. For a fish of 10 mm SL a minimum MSA value of 38′ was obtained by the formula of Neave (1984) whereas a maximum MSA value of 107′ was obtained using the formula given in Van der Meer and Anker (1984). The widely used formula of Tamura (1957) gave intermediate results, being approximately twice as high as Neave's values (i.e., 75′).

One critical parameter in the calculation of visual resolution is the focal length (f) of the lens, which is calculated as f = lens radius × 2.55. The value of 2.55 represents the ratio of eye diameter to lens diameter and is commonly referred to as Mathiessen's ratio. Some authors suggested a ratio of 2.36 to be more suitable for fish (Breck and Gitter 1983; Fernald 1985; Browman et al. 1990). Using 2.36 instead of 2.55 for Mathiessen's ratio shifts the regression line in Fig. 4 upwards (e.g., compare Neave and Browman et al. in Fig. 4). The result is a 25% higher MSA value by using the smaller Mathiessen's ratio of 2.36. However, we found that Mathiessen's ratio changed considerably with SL in *R. rutilus*, being even higher than 2.55 on average (Table 2).

The regressions depicting the behavioural estimates were different for the two species, with *R. rutilus* having higher MSA values than *P. flavescens* (Fig. 5). The two lines for the morphological estimates were actually similar for the two species but reflected the range of results obtained by applying different MSA formulas while using similar cone density and lens diameter (LD) values. The general trend was that in small fish, visual abilities estimated from behavioural performance were much worse (higher MSA values) than predicted from the morphological work. For a 10 mm SL fish, MSA values based on behavioural observations were 3–10 times higher than morphological estimates, depending on species and method of calculation. However, this factor diminished with increasing fish size. If a very conservative morphological method was employed (Van der Meer and Anker 1984), then similar MSA values were found at 35 mm SL for *P. flavescens* and at 100 mm SL for *R. rutilus*. After this point, behavioural performance was better (i.e., lower MSA values) than expected from the morphological work. Using an optimistic estimate of visual resolution from morphology (i.e., Neave 1984), MSA values were similar at 200 mm SL for *P. flavescens* and at 500 mm SL for *R. rutilus* (a size this species rarely reaches in nature). Above this size threshold, behavioural per-

© 1996 NRC Canada

Fig. 4. Minimum separable angle (MSA) versus standard length for a hypothetical size range of fish using lens diameter (x, in mm) and cone density (y, in number per 0.1 mm^2) calculated from the formulae given in Figs. 2 and 3. On the basis of x and y, MSA values were calculated using the following formulae from the literature without corrections for shrinkage: Van der Meer and Anker 1984, $MSA = 60/2^{½}(\pi/360)2.55(0.5x)(100y)^{½}$; Tamura 1957, $MSA = 1/(2.55 \times 0.5x)(0.2/y^{½})$; Browman et al. 1990, $MSA = \arcsin(1/((100y)^{½} \times 2.36 \times 0.5x)) \times 60$; Northmore and Dvorak 1979, $MSA = 6000/(20.5x \times y^{½})$; Neave 1984, $MSA = \arcsin(1/((100y)^{½} \times 2.55 \times 0.5x)) \times 60$. Curves were fitted to the resulting data points and drawn on a common log scale.

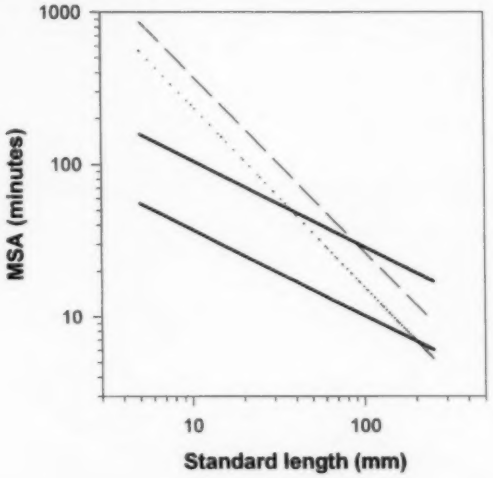


formance was always better than expected from cone density and lens diameters.

Discussion

Although the morphological bases of photopic vision were similar between the investigated species, their visual abilities involved in prey capture behaviours were significantly different. Two possibilities may account for this pattern: (*i*) there are morphological differences involved in prey detection capabilities that were not accounted for in this comparison, e.g., possible differences in the cone mechanism sensitive to short wavelengths (*P. flavescens*: Loew and Wahl 1991; *R. rutilus*: Avery et al. 1982; Douglas 1986) and (*ii*) the two species use different behaviours when searching for food, leading to differences in RD, e.g., cruising versus saltatory searching behaviour (sensu O'Brien et al. 1986). Behavioural differences in prey-searching tactics exist between the two species (J. Wanzenböck, unpublished data) but whether *P. flavescens* can be regarded as a true saltatory searcher will be assessed in future analyses. Anyhow, behavioural MSA differences between *P. flavescens* and *R. rutilus* have the same magnitude as behavioural differences found between *R. rutilus* and another cyprinid species, the bleak, *Alburnus alburnus* (Wanzenböck and Schiemer 1989). However, although the bleak and the yellow perch exhibit similar MSA values, prey-searching tactics used by the bleak are similar to those used by *R. rutilus*.

Fig. 5. Regression lines representing behaviourally determined MSA values (dotted line for *P. flavescens*, dashed line for *R. rutilus*) and morphologically determined MSA values (solid lines, representing the range of values resulting from using different formulae (see Fig. 4)).



Comparing our morphological estimates with values from the literature is extremely difficult, since a wide variety of visual resolution estimates are possible, depending on whether fixed or fresh lenses are used for measurements, whether fixed retinas are used to determine cone density and which consequent correction is used to account for shrinkage, where cell counts are made in different areas of the retina, and which formula is used to calculate MSA. All of the formulae we have used are much more sensitive to differences in LD (or radius) than to differences in cone density. For example, a 12% increase in MSA results from a 20% reduction in cone density, but MSA increases by 25% if LD is reduced by 20%. Therefore, it is more critical to get accurate measurements of LD. It is not appropriate to correct LD measurements taken from fixed samples by a simple shrinkage factor because lenses of small fish shrink proportionally more than lenses of larger fish owing to higher water content (Table 2).

We used different methods to assess cone density, i.e., counts were taken from the area temporalis of fresh retinas for *P. flavescens* and averaged counts from all over the retina of fixed material in *R. rutilus*. However, we found that cone density in the area temporalis is about 11% higher than the average cone density (Table 2), which is exactly the same value often used to account for shrinkage of the retina during fixation (Neave 1984; Browman et al. 1990). Therefore, the two factors cancel out and we arrived at similar cone density values for the two species examined (Fig. 3).

To illustrate the problems of comparing our morphological estimates with previously published values, consider the following examples. Some authors used cross sections through the whole eye to determine cone cell density (Blaxter and Jones 1967; Guma'a 1982; Neave 1984). Cone cell density cannot be reliably calculated from linear counts because there

© 1996 NRC Canada

are several possible ways to transect cones in the mosaic they form on the retina and get different numbers. Nevertheless, their cone cell density values, given as cells per 100 µm, are comparable to ours when their numbers are squared. Published values for the herring (*Clupea harengus*) (Blaxter and Jones 1967) and for Eurasian perch (*Perca fluviatilis*) (Guma'a 1982) are 7–10 cones/100 µm for fish of 200 mm length which, if squared, is close to our result of 50–100 cones/0.1 mm^2. Here we assume as well that shrinkage of the fixed retina cancels out counts from the unspecialized retina, which the above-mentioned authors used. However, estimates of MSA are either quite similar to ours (17–20′ for a 200-mm fish) when fresh lens measurements were used (Blaxter and Jones 1967: ~20′ for a 200-mm fish, Tamura's formula) or twice as high when fixed lenses were measured and shrinkage of lenses was not accounted for (Guma'a 1982: ~50′ for a 200-mm fish, Tamura's formula). Taking into account all of these problems, we conclude that the absolute values of morphologically derived visual resolution estimates reported in the literature are comparable to ours, not just the general trends (Blaxter and Jones 1967; Johns and Easter 1977; Northmore and Dvorak 1979; Guma'a 1982; Neave 1984; Browman et al. 1990; Pankhurst et al. 1993; Miller et al. 1993). The only exception is the data reported by Hairston et al. (1982) who found lower MSA values at corresponding fish sizes, both in behavioural (RD measurements) and morphological estimates of visual resolution for bluegill sunfish (*Lepomis macrochirus*).

The general pattern that expectations based on morphology overestimate visual resolution in small fish is in agreement with the literature. However, the ratio of morphologically to behaviourally derived estimates of MSA is not constant, changing drastically with fish size. This is corroborated by the findings of Hairston et al. (1982), Neave (1984), Pankhurst et al. (1993), and Miller et al. (1993). At a certain fish size, MSA values derived from the different methods are equal. Above this size, visual resolution determined behaviourally or physiologically (i.e., performance) is better than expected from cone density and lens diameter. Several features of the visual apparatus of fish change drastically during ontogeny, which may lead to this pattern. Pankhurst et al. (1993) suggested that the accommodative system is initially underdeveloped in larval fish and cannot compensate for potential refraction errors (myopia) caused by Mathiessen's ratio, which changes gradually while the fish mature to reach constant proportions in larger fish. This could lead to the performance of small fish being worse than predictions based on cone cell density and LD. The discrepancy becomes smaller during ontogeny facilitated by changes in cone mosaic, increasing complexity of inter-receptor contacts and synaptic feedback, changes within the optic tract, and the development of higher optic centres in the brain (Noakes and Godin 1988; Brandstätter and Kotrschal 1989; Kotrschal et al. 1991; Zaunreiter et al. 1991; Wahl et al. 1993; Pankhurst et al. 1993). The fact that performance becomes better than expected from cone cell density and LD data suggests the importance of these factors. It remains to be assessed whether or not the lifelong addition of rods contributes to this pattern and if so, under what circumstances. Perhaps further decisions regarding which calculations of MSA are most closely related to fitness will depend on the outcomes of experiments designed to test the importance of some of these parameters to the ultimate success and survival of the fish.

Acknowledgements

This study was made possible by an Erwin-Schrödinger-Stipendium awarded to J.W. by the Fonds zur Förderung der wissenschaftlichen Forschung, Austria, in 1990, which allowed a 1-year visit to the University of Guelph, Ontario, Canada. We are indebted to W. McFarland for his helpful comments during the preparation of the manuscript.

References

Avery, J.A., Bowmaker, J.K., Djamgoz, M.B.A., and Downing, J.E.G. 1982. Ultraviolet sensitive receptors in freshwater fish. J. Physiol. (London), **334**: 23–24.

Blaxter, J.H.S., and Jones, M.P. 1967. The development of the retina and the retinomotor responses in the herring. J. Mar. Biol. Assoc. U.K. **47**: 677–697.

Brandstätter, R., and Kotrschal, K. 1989. Life history of roach, *Rutilus rutilus* (Cyprinidae, Teleostei): a qualitative and quantitative study on the development of sensory brain areas. Brain Behav. Evol. **34**: 35–42.

Breck, J.E., and Gitter, M.J. 1983. Effects of fish size on the reactive distance of bluegill (*Lepomis macrochirus*) sunfish. Can. J. Fish. Aquat. Sci. **40**: 162–167.

Browman, H.I., Gordon, W.C., Evans, B.I., and O'Brien, W.J. 1990. Correlation between histological and behavioral measures of visual acuity in a zooplanktivorous fish, the white crappie (*Pomoxis annularis*). Brain Behav. Evol. **35**: 85–87.

Carpenter, S.R., and Kitchell, J.F. (*Editors*). 1993. The trophic cascade in lakes. Cambridge studies in ecology. Cambridge University Press, Cambridge, U.K.

Douglas, R.H. 1986. Photopic spectral sensitivity of a teleost fish, the roach (*Rutilus rutilus*), with special reference to its ultraviolet sensitivity. J. Comp. Physiol. A, **159**: 415–421.

Douglas, R.H., and Djamgoz, M. (*Editors*). 1990. Vision in fishes. Chapman and Hall, London.

Fernald, R.D. 1985. Growth of the teleost eye: novel solutions to complex constraints. Environ. Biol. Fishes, **13**: 113–123.

Grabner, K. 1990. Das Beutefangverhalten bei Rotaugen und Lauben. M.Sc. thesis, University of Vienna, Austria.

Guma'a, S.A. 1982. Retinal development and retinomotor responses in perch, *Perca fluviatilis* L. J. Fish Biol. **20**: 611–618.

Hairston, N.G., Li, K.T., and Easter, S.S. 1982. Fish vision and the detection of planktonic prey. Science (Washington, D.C.), **218**: 1240–1242.

Johns, P.R., and Easter, S.S. 1977. Growth of the adult goldfish eye. II. Increase in retinal cell number. J. Comp. Neurol. **176**: 331–342.

Kotrschal, K., Brandstätter, R., Gomahr, A., Junger, H., Palzenberger, M., and Zaunreiter, M. 1991. Brain and sensory systems. *In* Cyprinid fishes. Systematics, biology and exploitation. *Edited by* I.J. Winfield and J.S. Nelson. Chapman & Hall, London. pp. 284–331.

Lazzaro, X. 1987. A review of planktivorous fishes: their evolution, feeding behaviours, selectivities, and impacts. Hydrobiologia, **146**: 97–167.

Li, K.T., Wetterer, J.K., and Hairston, N.G. 1985. Fish size, visual resolution and prey selectivity. Ecology, **66**: 1729–1735.

Loew, E.R., and Wahl, C.M. 1991. A short-wavelength sensitive cone mechanism in juvenile yellow perch, *Perca flavescens*. Vision Res. **31**: 353–360.

Lythgoe, J.N. 1979. The ecology of vision. Clarendon Press, Oxford.

McFarland, W.N. 1992. The visual world of coral reef fishes. *In* The

© 1996 NRC Canada

ecology of fishes on coral reefs. Academic Press, New York. pp. 16–38.

Miller, T.J., Crowder, L.B., and Rice, J.A. 1993. Ontogenetic changes in behavioural and histological measures of visual acuity in three species of fish. Environ. Biol. Fishes, **37**: 1–8.

Munz, F., and McFarland, W.N. 1978. Evolutionary adaptations of fishes to the photic environment. *In* Handbook of sensory physiology. Vol. 5. No. 7. Springer-Verlag, Berlin. pp. 193–274.

Neave, D.A. 1984. The development of visual acuity in larval plaice (*Pleuronectes platessa* L.) and turbot (*Scophthalmus maximus* L.) J. Exp. Mar. Biol. Ecol. **78**: 166–175.

Noakes, D.L.G., and Godin, J.G.J. 1988. Ontogeny of behavior and concurrent developmental changes in sensory systems in teleost fishes. *In* Fish physiology. Vol. 11B. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 345–395.

Northcote, T.G. 1988. Fish in the structure and function of freshwater ecosystems: a "top-down" view. Can. J. Fish. Aquat. Sci. **45**: 361–379.

Northmore, D.P.M., and Dvorak, C.A. 1979. Contrast sensitivity and acuity of the goldfish. Vision Res. **19**: 255–261.

O'Brien, W.J. 1987. Planktivory by freshwater fish: thrust and parry in the pelagia. *In* Predation. *Edited by* C.W. Kerfoot and A. Sih. University Press of New England. pp. 3–16.

O'Brien, W.J., Evans, B., Howick, G.L., and Gregory, L. 1986. A new view of the predation cycle of a planktivorous fish, white crappie (*Pomoxis annularis*). Can. J. Fish. Aquat. Sci. **43**: 1894–1899.

Pankhurst, P.M., Pankhurst, N.W., and Montgomery, J.C.1993. Comparison of behavioral and morphological measures of visual acuity during ontogeny in a teleost fish, *Fosterygion varium*, Tripterygiidae (Foster, 1801). Brain Behav. Evol. **42**: 178–188.

Rahmann, H., Jeserich, G., and Zeutizius, I. 1979. Ontogeny of visual acuity of rainbow trout under normal conditions and light deprivation. Behaviour, **68**: 315-322.

Tamura, T. 1957. A study of visual perception in fish, especially on resolving power and accommodation. Nippon Suisan Gakkaishi, **22**: 536–557.

Van der Meer, H.J., and Anker, C.C. 1984. Retinal resolving power and sensitivity of the photopic system in seven haplochromine species (Teleostei, Cichlidae). Neth. J. Zool. **34**: 197–209.

Wahl, Ch.M., Mills, E.L., McFarland, W.N., and DeGisi, J.S. 1993. Ontogenetic changes in prey selection and visual acuity of the yellow perch, *Perca flavescens*. Can. J. Fish. Aquat. Sci. **50**: 743–749.

Walton, W.E., Hairston, N.G., and Wetterer, J.K. 1992. Growth-related constraints on diet selection by sunfish. Ecology, **73**: 429–437.

Walton, W.E., Easter, S.S., Jr., Malinoski, C., and Hairstone, N.G., Jr. 1994. Size-related change in the visual resolution of sunfish (*Lepomis* spp.). Can. J. Fish. Aquat. Sci. **51**: 2017–2026.

Wanzenböck, J., and Schiemer, F. 1989. Prey detection in cyprinids during early development. Can. J. Fish. Aquat. Sci. **46**: 995–1001.

Zaunreiter, M., Junger, H., and Kotrschal, K. 1991. Retinal morphology of cyprinid fishes: a quantitative histological study of ontogenetic changes and interspecific variation. Vision Res. **31**: 383–394.

© 1996 NRC Canada

Carbon and nutrient output from the Great Whale River (Hudson Bay) and a comparison with other rivers around Quebec[1]

C. Hudon, R. Morin, J. Bunch, and R. Harland

Abstract: The annual load and the composition of material exported to the coast of Hudson Bay were characterized and modelled over the hydrological cycle of the Great Whale River. Annual exportations of particulate inorganic matter (PIM), particulate organic matter (POM), and dissolved organic carbon (DOC) to Hudson Bay were estimated at 135 000, 21 000, and 90 000 t, respectively. PIM, POM, and DOC concentrations were positively related to discharge, as were concentrations of coarse (>1 mm), fine (53 μm – 1 mm), and very fine (0.7–53 μm) particulate organic matter. Of the three size fractions, very fine particulate organic matter exhibited the highest heterotrophic activity, the lowest C:N ratio, and the highest chlorophyll *a* concentration (1 $\mu g \cdot L^{-1}$) during summer. During the ice breakup period, 25–35% of the yearly PIM load, 23% of the POM load, 11% of the DOC load, and 5–14% of the dissolved nutrient load were exported to Hudson Bay. River waters were less concentrated in nutrients than Hudson Bay coastal waters. Comparison of DOC output with that of 46 other rivers draining the Precambrian Shield shows that, for a given runoff value, rivers flowing through tundra to Hudson and Ungava bays carried a specific DOC load about half that of rivers flowing through forested basins to the Gulf of St. Lawrence.

Résumé : La charge annuelle et la composition du matériel exporté sur la côte de la Baie d'Hudson ont été caractérisées et modélisées au cours du cycle hydrologique de la Grande Rivière de la Baleine. Les exportations annuelles de matière particulaire inorganique (PIM) et organique (POM) et de carbone organique dissous (DOC) vers la Baie d'Hudson ont été estimées à 135 000, 21 000 et 90 000 t, respectivement. Les concentrations de PIM, POM et DOC étaient positivement corrélées au débit, tout comme les concentrations de particules organiques de grande taille (>1 mm), de taille fine (53 μm – 1 mm) et de taille très fine (0.7–53 μm). Des trois fractions de taille, les particules très fines avaient le plus haut taux d'activité hétérotrophe, le plus faible rapport C:N ainsi que la plus forte concentration de chlorophylle *a* (1 $\mu g \cdot L^{-1}$) au cours de l'été. Pendant la période de la débacle printanière, 25–35% de la charge annuelle de PIM, 23% de la charge de POM, 11% de la charge en DOC et 5–14% de la charge en éléments nutritifs furent exportés vers la baie d'Hudson. Les eaux de la rivière étaient moins concentrées en éléments nutritifs que les eaux cotières de la baie. Une comparaison des apports de DOC avec 46 autres rivières drainant le Bouclier Précambrien montre que, pour une même valeur de ruissellement spécifique, les rivières coulant dans la tundra vers les baies d'Hudson et d'Ungava transportent une charge spécifique de DOC moitié moindre que celle des rivières coulant dans des bassins forestiers vers le Golfe du Saint-Laurent.
[Traduit par la Rédaction]

Introduction

Rivers from temperate latitudes are subjected to important seasonal variations in discharge that result in cyclic changes in the amount of terrestrial material carried by rivers to oceans. Particulate organic matter (POM) and dissolved organic carbon (DOC) represent important components of allochtonous river material (Schlesinger and Melack 1981; Mulholland and Watts 1982), which are processed biologically and degraded into smaller components during their transit downstream (Fisher 1977). River POM is broken down from coarse (CPOM; >1 mm) into fine (FPOM; 53 μm – 1 mm) and very fine (VPOM; 0.7–53 μm) particles and DOC (Boling et al. 1975; Naiman 1982). The microflora colonizing POM accelerate its degradation and modify its qualitative characteristics, such as particle size distribution and carbon, nitrogen, and phosphorus contents, and increase its palatability for stream insects (Richard et al. 1982). POM decomposition via biological activity releases inorganic nutrients and indirectly contributes to autochtonous carbon fixation by autotrophs. As the degradation of organic carbon continues, much of the remaining DOC is made up of refractory compounds such as humic and fulvic acids (Jackson 1975).

Seasonal variations in temperature determine the intensity

Received February 7, 1995. Accepted February 15, 1996.
J12760

C. Hudon,[2] R. Morin,[3] J. Bunch,[4] and R. Harland.[5] Arctic Biological Station, 555, boulevard Saint-Pierre, Sainte-Anne de Bellevue, QC H9X 3R7, Canada.

[1] Last contribution to the research program of the Arctic Biological Station, Sainte-Anne de Bellevue, Quebec.
[2] Present address: Environnement Canada, Centre Saint-Laurent, 105, rue McGill, Suite 700, Montréal, QC H2Y 2E7, Canada.
[3] Present address: Department of Fisheries and Oceans, P.O. Box 5030, Moncton, NB E1C 9B6, Canada.
[4] Present address: Department of Fisheries and Oceans, 200 Kent Street, Station 1182, Ottawa, ON K1A 0E6, Canada.
[5] Present address: Paprican, 570, boulevard Saint-Jean, Pointe Claire, QC H9R 3J9, Canada.

© 1996 NRC Canada

of biological activity and therefore set the rate of autochtonous production and of biological degradation of POM. Whereas discharge determines the quantity of particulate material, the qualitative characteristics of particles should vary with temperature. Therefore, seasonal variations in discharge and in temperature must be considered simultaneously to understand the yearly cycle of particulate and dissolved materials within river systems.

Over the last decades, the seasonal regime of several large rivers flowing into the James and Hudson bays has been altered to generate hydroelectric power. Damming alters flow regimes and curtails the natural extremes of discharge, around which biological communities have evolved. In addition to the effects of individual projects, their cumulative impact on the overall productivity of coastal areas of Hudson Bay is a source of concern (Bunch and Reeves 1992). This possibility can only be examined through quantitative, year-round assessment of the quantity and quality of the materials exported to the coastal waters by the large rivers draining this territory (Burton 1988).

This study provides an estimate of the yearly outflow of particulate and dissolved material from the Great Whale River to coastal Hudson Bay. It also documents the temporal variations in the qualitative characteristics of the exported materials in relation to the seasonal changes in discharge and temperature. This basic information permits a critical examination of the importance of the potential contribution of the Great Whale River to Hudson Bay coastal production. The yearly output of DOC by the Great Whale River to Hudson Bay is then put into perspective, by a comparison with 46 other large rivers draining the Precambrian Shield. This exercise provides an additional tool for assessing the impact of large-scale environmental changes on the potential productivity of coastal waters.

Materials and methods

Description of the study area

The Great Whale River (mean annual discharge 666 $m^3 \cdot s^{-1}$) is typical of the large rivers draining central Quebec into James and Hudson bays, ranking fifth in importance after the LaGrande, Nottaway, Eastmain (before impoundment), and Rupert rivers. The Great Whale River (Fig. 1) flows 416 km from an elevation of 427 m, draining an area of 42 735 km^2. The river flows on the rugged, granitic bedrock of the Canadian Precambrian Shield, which supports extensive bogs and low-gradient streams. The vegetation of the upper watershed is an assemblage dominated by spruce (*Picea* spp.), tamarack (*Larix laricina*), and trembling aspen (*Populus tremuloides*) interspersed with areas of lichen and peatland bogs. Balsam poplar (*Populus balsamifera*), sweet gale (*Myrica gale*), and speckled alder (*Alnus rugosa*) are found in the riparian areas. The vegetation of the lower 10 km of the river is characteristic of the transition zone of the forest–tundra (Ministère des Forêts du Québec 1991).

The seasonal discharge of large northern temperate rivers follows a pattern of high spring and autumn discharge, alternating with flow reductions in summer and winter (Ministère de l'Environnement du Québec 1991). The Great Whale River discharge closely follows the seasonal cycle of temperature and precipitation. Total mean (1940–1970) annual precipitation is 636 mm, of which 39% falls as snow between October and May (Société d'énergie de la Baie James 1978). Maximum water temperature is 18°C in August, with intermediate discharge values (400–800 $m^3 \cdot s^{-1}$). Cooling weather and heavy rains in the fall prompt a drop in temperature and a rise in discharge (650–1100 $m^3 \cdot s^{-1}$) from September to mid-October. Average monthly temperature (<1°C) and discharge are lowest during winter (100–300 $m^3 \cdot s^{-1}$). The Great Whale River has a 1 m thick ice cover from approximately mid-November to late May. Spring snowmelt generates a dramatic 2-week peak of river discharge, during which the ice cover breaks up and about 10% of the annual discharge occurs. However, the magnitude (1040–2370 $m^3 \cdot s^{-1}$) and timing (between May 10 and June 17) of the spring freshet vary among years (1959–1969 data; Environment Canada 1992).

Sampling

Sampling took place from June 13, 1990, to August 9, 1991, at a station located 2.5 km from the mouth of the Great Whale River (55°16.35′N, 77°44.67′W) and at a depth of 1 m. This location was within the freshwater tidal zone. Water was mixed throughout the channel, as was shown by analysis of variance of replicate DOC and particulate organic carbon (POC) samples collected at the surface, at mid-depth, and near the bottom at four equidistant sites across the river (see also Hudon 1994). The site was close to the laboratory facilities of the Centre d'études nordiques (Université Laval) in Kuujjuarapik, allowing the processing of samples shortly (<2 h) after collection. Preserved and frozen samples were further analyzed after shipment to the Arctic Biological Station (near Montréal, Que.).

Daily discharge (cubic metres per second) values reported from upriver gauging stations (Ministère de l'Environnement du Québec 1991) were corrected to account for the entire watershed. Water temperature was recorded hourly using a thermograph located in the municipal pumping well; well water temperature was within 1°C of river temperature at 1 m.

Yearly load of dissolved and particulate material

Eight 5-mL samples of river water were filtered (53 μm) and frozen until analyzed. Dissolved reactive phosphorus (DRP; detection limit 1 μg $P \cdot L^{-1}$) (Strickland and Parsons 1972), ammonium (NH_4; detection limit 10 μg $N \cdot L^{-1}$) (Technicon Industrial Systems 1973) and nitrate–nitrite (NO_3; detection limit 1 μg $N \cdot L^{-1}$) (U.S. Environmental Protection Agency 1979) analyses were carried out using a Technicon Autoanalyzer II.

One 9-L sample was taken at a 1 m depth every 1–3 weeks. Total POM was determined by six 1-L replicates filtered through pre-ashed, pre-weighed, 47 mm diameter glass-fibre filters (Gelman GF/F). Filters were weighed after drying (70°C for 24 h) and ashing (550°C for 4 h) to evaluate the mineral (particulate inorganic matter (PIM); ash weight) and organic (POM; ash-free dry weight) components of particulate matter (Wetzel and Likens 1991). From the same sample, 2- to 100-mL subsamples were filtered through pre-ashed 25 mm diameter GF/F filters to estimate DOC and POC according to the method outlined in Bunch (1987). The percentage of organic carbon contained in POM was then calculated and used to estimate the carbon (POC plus DOC) load into Hudson Bay.

Monthly and annual loads of PIM, POM, and DOC were modelled from regressions between measured concentrations and corresponding discharge values. For each variable, linear, log-linear, and polynomial (up to fourth order) regression models were examined. The model used to predict daily loads was selected on the bases of maximized r^2, highest r^2 increment (for successive polynomial orders), and detrended residuals.

Seasonal changes in the quality of particulate matter

To contrast the seasonal changes in the characteristics of POM, more detailed analyses were carried out on each size fraction at spring ice breakup, midsummer low flow, autumn, and winter. Spring sampling was conducted in June 1990, 3 weeks after ice breakup, and in May–June 1991, within 4 days of ice breakup. Winter sampling was conducted in January 1991 but was repeated in March owing to equipment malfunction.

Seston was separated into three classes of particle diameter: CPOM (>1 mm), FPOM (53 μm – 1 mm), and VPOM (0.7–53 μm).

© 1996 NRC Canada

Fig. 1. Location of the Great Whale River and of 46 Quebec rivers used for comparison of yearly area-specific DOC exports. Bold lines indicate the limits of hydrographic drainage basins. Major rivers forming sub-basins, such as the Saguenay (33), Ottawa (42), and St. Lawrence (47) rivers, are identified by name. River number refers to the information detailed in the Appendix.

These categories are similar to those used in other studies (Boling et al. 1975; Naiman 1982).

CPOM and FPOM were obtained by pumping water through sieves or by passive collection with drift nets. In the open-water period, between 3000 and 6000 L of river water was pumped through 1-mm and 53-μm mesh sieves. Six prefiltered (53-μm mesh) 1-L samples were filtered through pre-ashed and pre-weighed Gelman GF/F glass fibre filters to measure VPOM. When larger (approximately 700 000 L) filtration volumes were required to collect sufficient amounts of CPOM, a 1-mm plankton net (0.5 m diameter, 1 m length) was used. At the laboratory, macroinvertebrates were removed from the CPOM and FPOM samples and the samples were frozen whole in Whirl Paks. Samples were weighed after drying (70°C for 24 h) and ashing (550°C for 4 h) to calculate ash-free dry weight (AFDW).

During winter, CPOM and FPOM were collected 1 m below the ice using a 0.5 m diameter rim fitted with a 1 mm mesh net (1 m long) inside a 53-μm mesh (1.5 m long) plankton net. Filtered volume varied typically between 3000 and 24 000 L, estimated from a flowmeter (General Oceanics, model 2030).

Each seston size fraction was examined using scanning electron microscopy and characterized by its pigment composition, C:N ratio, organic N content, bacterial counts, and activity. Values for CPOM and FPOM were expressed in relation to organic contents (per gram AFDW), since particulate matter abundance varies seasonally, as does discharge. Samples (100 mL) of river water and 1:10 suspensions of each size fraction were preserved with 0.8% glutaraldehyde and chilled until filtration. Air-dried filters were coated with gold–palladium and examined (30 – 10 000 ×) by scanning electron microscopy.

Chlorophyll *a* and phaeopigment concentrations in CPOM and FPOM were determined by the method outlined in Strickland and Parsons (1972), modified for macrophytes. Pigments were extracted from approximately 1 g of particulate material thawed under dark conditions to a temperature of 1–2°C. Pigments were extracted in 20 mL of 90% acetone at 4°C in the dark for 24 h, prior to spectrophotometric measurements of absorption before and after acidification (Jeffrey and Humphrey 1975). After pigment extraction, the dry and ashed weights of CPOM and FPOM residues were measured to

© 1996 NRC Canada

Table 1. Nutrient concentrations (μM, mean ± SD) and number of measurements (in parentheses) in the Great Whale River.

Nutrient	June 1990	August 1990	October 1990	January 1991	March 1991	May–June 1991
NO_3	1.0±0.1 (8)	0.5±0.1 (4)	0.2±0.1 (8)	1.3±0.01 (3)	2.1±0.1 (4)	1.1±0.2(10)
NH_4	0.5±0.1 (4)	0.8±0.4 (2)	1.0±0.2 (4)	1.6±0.4 (2)	1.9±0.1 (2)	1.2±0.2 (5)
N_{tot}	3.6±0.9 (7)	2.9±0.5 (4)	2.4±0.9 (8)	3.6±0.7 (2)	4.3±0.5 (4)	3.3±1.1 (10)
DRP	0.17±0.05 (6)	0.06±0.01 (3)	0.08±0.02 (8)	0.08±0.03 (3)	na	na

Note: DRP, dissolved reactive phosphorus; na, results not available.

express pigment concentration per gram of organic matter. Pigment extraction for VPOM followed standard procedures for plankton (Strickland and Parsons 1972).

Carbon and nitrogen contents of particulate matter were measured with an elemental analyzer (Perkin Elmer model 240) following the method of Stainton et al. (1977). Total Kjeldahl nitrogen in CPOM and FPOM was assessed following the method of the American Public Health Association (1981).

Free-living bacteria were enumerated in triplicate from 1-mL samples of the 53-μm filtrate (VPOM) preserved in 0.8% glutaraldehyde. Samples were filtered on prestained 0.2-μm Nuclepore filters (Watson et al. 1977) with acridine orange, and bacteria were enumerated from 10 random 1000× fields.

Heterotrophic potential was measured using radiolabelled glutamic acid (193 mCi·mmol C^{-1}; ^{14}C; 1 Ci = 37 GBq). Weighted quantities of freshly collected CPOM and FPOM were resuspended in 500 mL of sterile river water. VPOM activity was measured from the 53-μm filtrate. For each fraction, aliquots of the whole and diluted (1:10 and 1:100) resuspensions were incubated in chilled, sterile 55-mL screw-cap bottles. Replicate bottles were incubated in the dark at a temperature ±3°C that of the river. Incubation was stopped after 0, 1, 2, 3, 4, and 6 h by filtration through a 0.45-μm Millipore filter. Activity was measured by liquid scintillation counting.

Comparison with other rivers

The DOC measured for the Great Whale River was included in a set of 55 average DOC concentrations reported for 47 rivers (see Appendix). Seven duplicate values (aux Outardes, Manicouagan, Trinité, Moisie, Musquaro, and Great Whale rivers) and one triplicate value (Matamek River) were obtained from different studies and were kept as separate DOC estimates. The large rivers (watershed >500 km^2) selected for comparison all flow through the Precambrian Shield (Quebec) and belong to five hydrographic drainage basins (Fig. 1). This included 4 rivers flowing into southeastern Hudson Bay, 6 rivers flowing into Ungava Bay, 25 rivers flowing into the Gulf of St. Lawrence, and 11 rivers draining into the Gulf of St. Lawrence via the St. Lawrence and Ottawa rivers (Appendix). The St. Lawrence and Ottawa rivers were also included in the set, even though they do not exclusively drain the Precambrian Shield.

The relationships between watershed characteristics (hydrographic basin, human activities, surface area, discharge, runoff, latitude, and vegetation) and DOC concentration and area-specific DOC exports were initially assessed using nonparametric (Spearman rank, r_s) correlations. Watershed surface area (square kilometres; Ministère des Richesses Naturelles du Québec 1969) and mean annual discharge (cubic metres per second; Environment Canada 1992) were used to calculate river runoff (millimetres per year) and area-specific DOC exports (tonnes per square kilometre in a year) for each data point. Human activities (logging, hydroelectric development, agriculture, urbanization) likely to affect river DOC load were also assessed for degree of impact on a scale of 0 (pristine watershed) to 4 (highly utilized watershed). The vegetation of each watershed was classified into seven categories, ranging from 0 (arctic tundra) to 6 (deciduous forest) (Ministère des Forêts du Québec 1991). The predictive relationship between river runoff and area-specific DOC exports was then obtained by linear regressions.

Results

Physical and chemical characteristics

Daily spring discharge in the Great Whale River during ice breakup peaked at 2100 and 1600 $m^3 \cdot s^{-1}$ for 1990 and 1991, respectively; these values were close to the maximum recorded for the previous 5 years. The average daily water temperature rose from 8°C at the beginning of sampling (June 1990) to a maximum of 18°C in August and declined steadily until it reached 0°C (November 1990 to late May 1991). Water temperature increased sharply from 0 to 12°C within 2 weeks of ice breakup.

Nutrient levels of the Great Whale River were typical of the oligotrophic waters of the Canadian Shield, characterized by low concentrations of inorganic nitrogen (NO_3 and NH_4) and DRP (Table 1). Nitrogen concentrations (all forms) were slightly higher in January and March (under ice cover) than during the ice-free period. DRP concentrations were near the detection limit during summer and were slightly higher during spring.

The amount of reactive nitrogen exported to Hudson Bay was on the order of 4.7 $t \cdot day^{-1}$ in May–June (using a freshet discharge of 2000 $m^3 \cdot s^{-1}$) and 0.8 $t \cdot day^{-1}$ in August (using a minimum summer discharge of 500 $m^3 \cdot s^{-1}$). Daily loads of DRP carried to the bay during the same periods equaled 0.9 and 0.09 $t \cdot day^{-1}$, respectively.

Yearly load of particulate and dissolved material

Peaks in the concentrations of PIM, POM, and DOC followed the hydrological cycle (Fig. 2). PIM concentrations were low for most of the hydrological cycle, except for a brief, dramatic increase during spring freshet. POM and DOC concentrations were slightly higher during the open water season than under ice cover; however, they also showed a sharp increase with the spring rise in discharge. Late winter (March) concentrations of PIM, POM, and DOC increased by factors of 30, 4, and 1.5 in spring, respectively. For the three categories, the highest concentrations coincided with the period of rapidly rising discharge; particle concentration (especially PIM) decreased sharply thereafter even though discharge remained high.

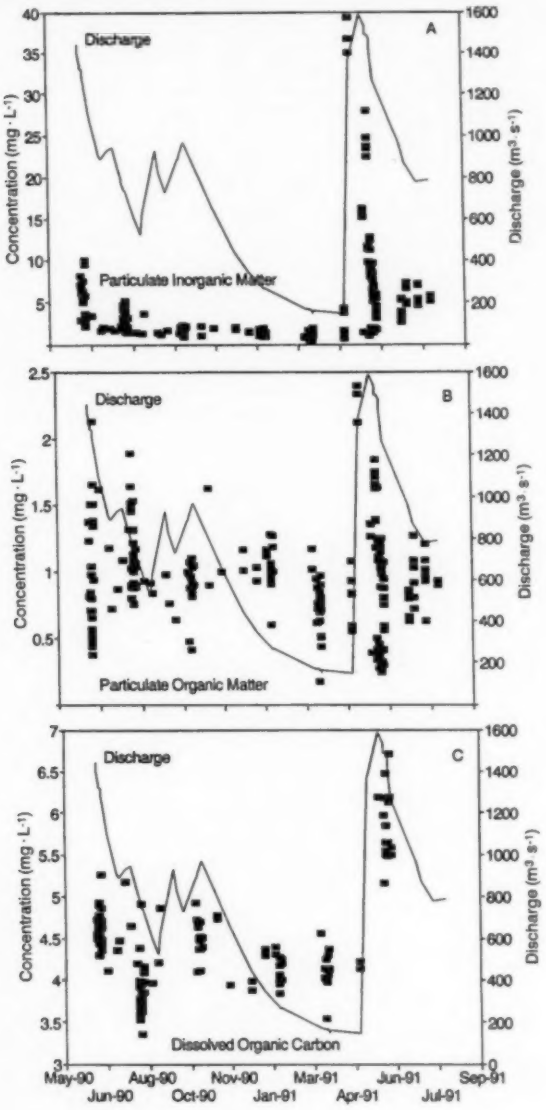
Regression models of the daily concentrations of PIM, POM, and DOC yielded the following predictive equations with discharge (Q; $m^3 \cdot s^{-1}$):

$$\text{PIM } (g \cdot L^{-1}) = -2.4184 + 0.0284Q - 4.543 \times 10^{-5} Q^2 + 0.002 \times 10^{-5} Q^3$$
$$p < 0.001,\ r^2 = 0.52,\ n = 212$$

$$\text{POM } (g \cdot L^{-1}) = 0.2295 + 0.0058Q - 0.7570 \times 10^{-5} Q^2 + 0.029 \times 10^{-5} Q^3$$
$$p < 0.001,\ r^2 = 0.39,\ n = 212$$

© 1996 NRC Canada

Fig. 2. Seasonal variations of (A) PIM, (B) POM, and (C) DOC concentrations (mg·L^{-1}) and of river discharge (m^3·s^{-1}; right-hand *y* axis) in the Great Whale River.



$$DOC\ (mg{\cdot}L^{-1}) = 4.4007 + 0.0016Q + 0.1587 \times 10^{-5}\ Q^2$$
$$p < 0.001,\ r^2 = 0.58,\ n = 139$$

Of the three, the relationship predicting DOC concentration was the strongest, with 58% of the total variance explained by discharge. Polynomial regressions were selected for their ability to accurately model the steep rise in concentrations at the time of spring freshet and produce detrended residuals. Estimation of extreme, but short-lived, springtime concentrations had a strong impact on calculation of yearly load (Table 2), representing 25–35% of the yearly value for PIM. The regression between PIM concentration and discharge was influenced by the high values recorded in the spring of 1991, when sampling took place only 4 days after ice breakup in the river.

Table 2. Monthly load of PIM, POM, and DOC (10^3 t), as estimated from polynomial regressions (see text) with river discharge, between June 1990 and July 1991.

	PIM	POM	DOC
June	62	5	20
July	8	2	10
August	6	2	7
September	7	2	9
October	9	2	10
November	6	2	7
December	4	1	5
January	2	0.7	3
February	1	0.3	2
March	1	0.3	2
April	0.4	0.2	2
May	29	3	13
June	21	3	15
July	8	2	10
Total spring 1990 (June–May)	135	21	90
Total spring 1991 (July–June)	94	19	85

Note: A comparison of yearly total outflow including the spring of 1990 (June 1990 – May 1991) and the spring of 1991 (July 1990 – June 1991) is presented.

During the remainder of the open water season, PIM and DOC loads were in the same order of magnitude, decreasing by half between July and December (Table 2). Under the ice, monthly loads of DOC (2000–5000 t·month^{-1}) accounted for the bulk (50–70%) of the material transported by the river. Yearly PIM, POM, and DOC loads to Hudson Bay varied by 17, 5, and 3%, respectively, from estimates made using values from the spring of 1990 or spring of 1991 (Table 2).

The average carbon content of POM was 51%. This value converts POM to a yearly POC load of 11 000 t·year^{-1} and a total organic carbon (TOC) load on the order of 10^5 t·year^{-1} to Hudson Bay.

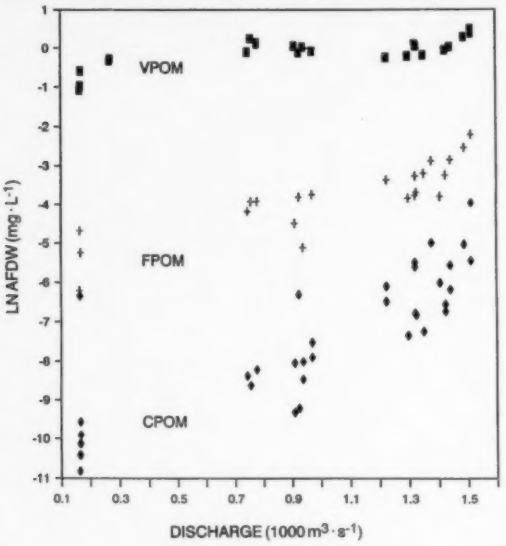
Seasonal changes in quality of POM

Scanning electron microscopic examination of suspended matter revealed the presence of fragments of terrestrial macrophytes (pollen, seeds, and decaying vegetative parts) and aquatic organisms (bacteria, algae, protozoans, and insects) amongst mineral particles (clay aggregates and sand grains). Filamentous green and blue-green algae as well as several diatom taxa (*Asterionella* sp., *Melosira* sp., *Tabellaria fenestrata*, *Tabellaria flocculosa*, *Fragilaria* sp., and *Synedra* sp.) were commonly identified. Planktonic colonies of *Dynobryon* sp. as well as sessile protozoan colonies were observed.

The three POM size fractions differed primarily by the relative abundance of their components but did not change markedly with seasons. The VPOM (0.7–53 µm) was characterized by planktonic diatoms, bacteria, and clay aggregates. The FPOM (53 µm – 1 mm) consisted of fragments of terrestrial macrophytes (leaves, wood fibers) in various stages of degradation, showing colonization by bacteria and attached diatoms. Clay aggregates, fecal pellets, and insect fragments were also observed. The CPOM (>1 mm) exhibited larger plant fibers

© 1996 NRC Canada

Fig. 3. Concentrations (ln mg·L^{-1} ash free dry weight (AFDW)) of CPOM, FPOM, and VPOM as a function of river discharge (m^3·s^{-1}).



and decaying vegetation entangled with filamentous algae, but few mineral particles.

VPOM constituted the most important fraction, 200–4000 times higher than CPOM and 13–170 times higher than FPOM. FPOM was 8–130 times higher than CPOM. The relation of particle concentration to discharge differed markedly between fractions (Fig. 3). CPOM was most strongly related to discharge, whereas VPOM concentration increased little with discharge.

During the open water period, CPOM and FPOM contained 2–5% (organic weight) nitrogen (total Kjeldahl). For both size fractions, August values were higher than in June 1990 and May 1991 (Table 3).

The C:N ratio of all fractions was lowest in August. The C:N ratio of VPOM increased by 2.5 times from a mean value of 8 in summer to 20 in late winter (Table 4). Comparable seasonal readings were not always available for larger size fractions; however, in August, October, and June 1991, C:N ratios tended to decrease with size of POM fractions.

Chlorophyll *a* and phaeopigment concentrations exhibited different seasonal variations in the three POM size fractions (Fig. 4). For the two larger size fractions (Figs. 4A and 4B), the results were presented per gram of AFDW rather than per litre of water to eliminate the concurrent effect of seasonal changes in POM concentration (related to discharge) on pigment concentrations. Pigment concentrations in CPOM and FPOM reflected the extent of microalgal colonization (chlorophyll *a*) on the surface of decaying terrestrial vegetation (phaeopigment). For both size fractions, the chlorophyll *a* concentration was always higher than the phaeopigment concentration; the two pigments followed similar temporal changes in concentration, resulting in a stable (<1) pigment ratio through time.

In contrast, the pigment concentrations in VPOM followed a clear seasonal cycle (Fig. 4C): they were highest in spring and summer (about 1 μg·L^{-1}), declined in October, and reached their lowest levels in winter (about 0.2 μg·L^{-1}). Chlorophyll *a* concentration in the VPOM fraction was expressed per litre of river water, since this fraction likely represented phytoplankton biomass. Since phaeopigment concentration remained stable through time, the ratio of phaeopigment to chlorophyll *a* fluctuated markedly, indicating that the physiological state of the phytoplankton was highest in August (ratio <1) and lowest in winter (ratio >1). The VPOM fraction accounted for the bulk of the chlorophyll *a* present in the Great Whale River: for comparison, maximum summer chlorophyll *a* concentrations in CPOM, FPOM, and VPOM equalled 0.002, 0.04, and 1 μg·L^{-1}, respectively.

Table 3. Percentage of nitrogen (total Kjeldahl) in the coarse (CPOM) and fine particulate organic matter (FPOM) during different periods of the hydological cycle in the Great Whale River.

Particle size	June 1990	August 1990	May 1991
CPOM (>1 mm)	na	5±0.3 (6)	2±0.001 (5)
FPOM (0.53 μm – 1 mm)	4±0.6 (9)	4±0.6 (2)	4±1 (8)

Note: Values are given as the mean ± SD, with the number of samples in parentheses. na, results not available.

Bacterial counts in the smallest POM fraction (VPOM) showed seasonal variations linked with discharge and temperature regimes (Fig. 5A). Free bacteria were most abundant in the spring and summer and less numerous during winter months. Estimates of bacterial carbon content (1.43×10^{-13} g/bacteria; Bratbak and Dundas 1984; Bunch and Harland 1990) and of bacterial density (about 1.5×10^9 bacteria/L) during the June 1990 freshet (monthly average discharge 1350 m^3·s^{-1}) indicate that they represented an outflow of 376 t of carbon (16% of the POC) over the 15 days following ice breakup. Daily bacterial carbon load to Hudson Bay varied from 25 t·day^{-1} in spring to about 1 t·day^{-1} in winter.

The rate of glutamic acid uptake varied seasonally and between POM size fractions (Fig. 5B). For all size fractions, bacterial assimilation of glutamic acid (per gram AFDW) was higher in August than in winter months, with a sharp spring rise for VPOM. Assimilation was constantly higher in VPOM than in FPOM and CPOM. Assimilation rate in VPOM followed the same seasonal pattern as the number of free bacteria in that fraction (Fig. 5). Overall, bacterial abundance and assimilation rates followed the seasonal temperature and discharge regime.

Comparison with other large Precambrian Shield rivers

Per unit of watershed area, the amount of DOC exported annually by the Great Whale River is 2.09 t·km^{-2}·year^{-1} (Appendix). The relative seasonal stability of DOC concentrations in the Great Whale River suggested that the average value obtained from a limited number of data could provide a reasonable estimate of yearly DOC load for a multiriver, large-scale comparison. This approach further assumed that differences in DOC concentrations owing to differences in methods (Koprivnjak et al. 1994) would be smaller than those resulting from watershed characteristics. Duplicate values obtained for the same watershed from different studies were retained to assess this factor.

The results of the correlations among watershed characteristics reflected the known changes occurring over the terri-

© 1996 NRC Canada

Table 4. Carbon/nitrogen ratio in the coarse (CPOM), fine (FPOM), and very fine (VPOM) particulate organic matter during different periods of the hydological cycle in the Great Whale River.

Particle size	June 1990	August 1990	October 1990	January 1991	March 1991	June 1991
CPOM (>1 mm)	na	11±0.1 (3)	21±2 (3)	na	na	15±0 (1)
FPOM (53 µm – 1 mm)	12±1 (5)	11±0 (1)	12±0 (1)	na	na	14±1 (4)
VPOM (0.7–53 µm)	na	8±1 (4)	11±1 (7)	22±1 (3)	20±1 (7)	12±0 (1)

Note: Values are given as the mean ± SD, with the number of samples in parentheses. na, results not available.

Fig. 4. Seasonal variations (mean ± SD) in chlorophyll *a* (solid line) and phaeopigments (broken line) in (A) CPOM (mg·g^{-1} AFDW), (B) FPOM (mg·g^{-1} AFDW), and (C) VPOM (mg·L^{-1}).

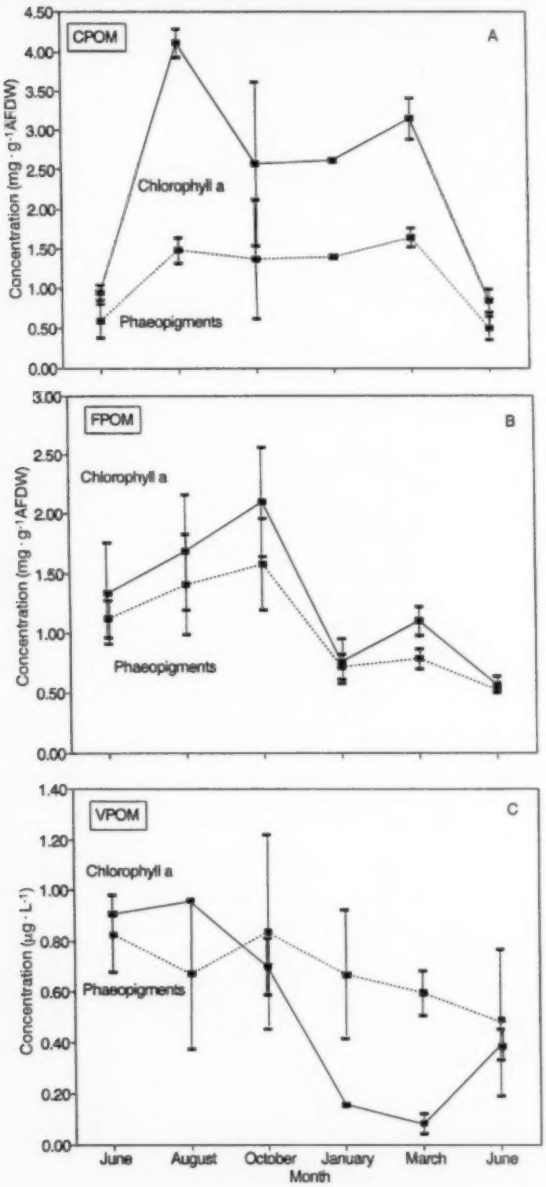
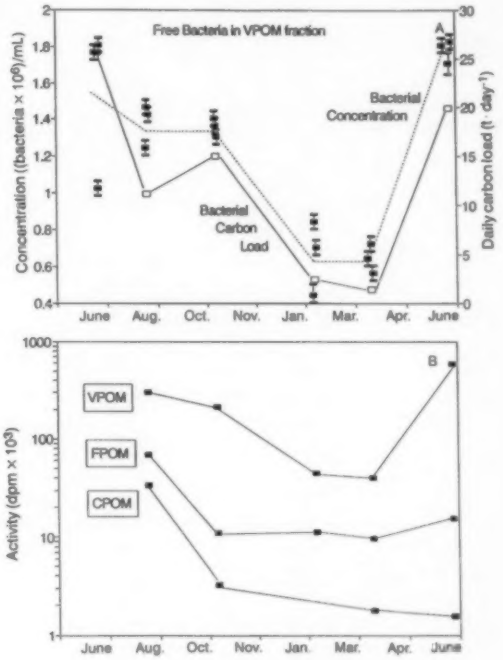


Fig. 5. Seasonal variations in (A) concentration of free bacteria (mean ± 95% CI) in VPOM, and bacterial carbon load exported daily to Hudson Bay (right-hand *y* axis) and (B) heterotrophic activity (disintegrations per minute, ×10^3) in the three POM size fractions.



tory under study: strong negative correlations were observed between vegetation and latitude ($r_s = -0.90$, $p < 0.001$) and hydrographic basin ($r_s = -0.96$, $p < 0.001$). The concentration of human activities in the Ottawa and St. Lawrence river basins was reflected by the correlations between human activities and latitude ($r_s = -0.72$, $p < 0.001$) and hydrographic basin ($r_s = 0.68$, $p < 0.001$) but did not correlate with specific DOC concentration ($r_s = 0.07$, $p > 0.05$) or with area-specific exports ($r_s = -0.19$, $p > 0.05$) (Table 5).

DOC concentrations were strongly related to vegetation ($r_s = 0.48$, $p < 0.001$) and reflected the simultaneous influence of hydrographic basin and latitude (Table 5). Area-specific DOC exports were strongly correlated with runoff ($r_s = 0.83$, $p < 0.001$) and showed the persistent effect, albeit to a lesser extent, of hydrographic basin and vegetation. These results suggest that the amount of DOC exported annually per unit of surface area is determined by the amount of water flowing through the watershed and its type of vegetation. The differ-

© 1996 NRC Canada

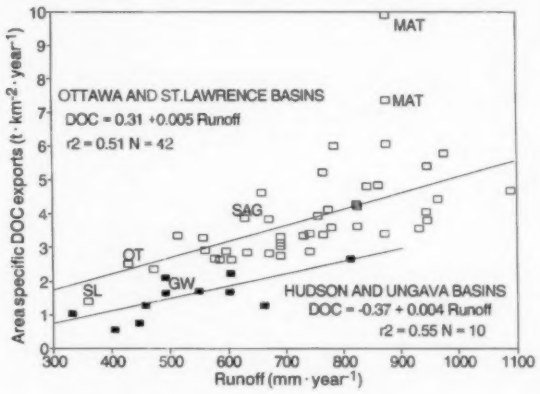
Table 5. Spearman rank correlation coefficients (r_s) between DOC concentration, area-specific exports of DOC, and river basin characteristics, for the 55 data points (47 rivers + 8 duplicate values) obtained from the literature (Appendix).

	DOC concentration ($mg \cdot L^{-1}$)	DOC exports ($t \cdot km^{-2} \cdot year^{-1}$)
Hydrographic basin	−0.45***	−0.29*
Human influence	0.07	−0.19
Watershed surface	−0.26	−0.63***
Mean discharge	−0.26	−0.57***
Runoff	0.27*	0.83***
Latitude	−0.38**	−0.17
Vegetation	0.48***	0.27*

Note: The associated p value is indicated for each coefficient: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. The other values were not significant.

ence in specific DOC load between rivers flowing through southern forested watersheds and through northern tundra watersheds was clearly observed when all data points were plotted as a function of river runoff (Fig. 6). For a given runoff value, rivers flowing to the Gulf of St. Lawrence carried a specific DOC load consistently higher than rivers flowing to Hudson and Ungava bays.

Fig. 6. Relationships between river runoff ($mm \cdot year^{-1}$) and area-specific DOC exports ($t \cdot km^{-2} \cdot year^{-1}$) for 47 Quebec rivers, divided into two groups of hydrographic drainage basins (Appendix). Rivers draining into Ungava–Hudson bays (solid symbols) are distinguished from rivers draining into the Gulf of St. Lawrence (open symbols). Individual data points for the Great Whale (GW), Ottawa (OT), St. Lawrence (SL), and Saguenay (SAG) rivers are presented, and two Matamek River outliers (MAT) are also shown. The two Matamek outliers were not used to calculate the regression for the Ottawa and St. Lawrence basins.



Discussion

Northern rivers are subjected to wide seasonal variations in discharge and temperature, which greatly influence the physical and biological processes regulating the concentrations and the fate of dissolved and particulate material. The assessment of the load of dissolved and particulate matter in the river over the yearly cycle allows us to assess the relative importance of the spring ice breakup in the yearly cycle. We will also compare the nutrient and carbon concentrations in river and coastal Hudson Bay waters, to evaluate the seasonal contribution of the terrestrial to the marine environment. The relative importance of those contributions is put in perspective by comparing the DOC output from the Great Whale River with that of other large rivers draining the Precambrian Shield.

Seasonal changes in the Great Whale River

The conditions prevailing during the warm, open water period of summer and fall are fairly well documented for large northern rivers (see reviews by Dodge 1989; Degens et al. 1991). Low rainfall and moderate discharge generate little shoreline erosion and entrainment of mineral particles, which results in low concentrations in the river (Fig. 2). Nutrients were also in low concentrations, in part owing to the inherently low supply in the Precambrian Shield bedrock and to the biological demand of autotrophs. The phytoplanktonic component of VPOM also reached its highest biomass during summer (Fig. 4), which coincided with a low flushing rate, high water clarity, and long period of daily illumination.

Organic particles of terrestrial origin were also in good supply during summer and fall, and exhibited high, but variable concentrations (Fig. 2). POM from terrestrial vegetation is known to provide highly significant amounts of organic matter to streams, both as POM and as leached dissolved organic matter (DOM) (Fisher and Likens 1973; Bilby and Likens 1980). The microbial activity related to this terrestrial plant debris was highest during warm summer months (Fig. 5B); CPOM and FPOM degradation was likely favored by their colonization by bacteria, fungi, and microalgae (Fig. 4). Such colonization coincided with the highest percentage of nitrogen contents and lowest C:N ratio in large particles, which likely increased their palatability for insects and grazers (Cummins 1973; Richard et al. 1982; Petersen et al. 1989). Nevertheless, the smallest size fraction (VPOM) remained by far the most abundant (Fig. 3), with the highest chlorophyll *a* concentration (Fig. 4C) and the highest heterotrophic activity (Fig 5B). The lower refractory carbon contents of VPOM in than of larger size fractions were further shown by VPOM's low C:N ratio (8.5 in August; Table 4).

The year-round occurrence of high concentrations of VPOM and DOC could result from their high supply through biological processes during the periods of low summer flow. Summer and fall represent a period of high biological activity in the river and its watershed: fine particles and DOM were likely in constant supply to the river system via the biological degradation of coarse particles as well as directly supplied to the river through runoff of terrestrial production.

Winter marked the onset of entirely different conditions for river organisms: river discharge, temperature, and illumination declined to their year-round minima. Isolation of river waters under 1 m of ice and snow minimized the inflow of terrestrial PIM and POM, both of which reached their lowest concentrations (Fig. 2). DOC concentration also decreased in winter, but to a lesser extent (Table 2), owing to the continuing degradation of POM by persistent, low-level microbial activity (Fig. 5). This period of low illumination coincided with a reduction in algal biomass, which was evident from the low chlorophyll *a* content of all POM size fractions (Fig. 4), from the increase in C/N ratio (Table 4), and from the decrease in per-

© 1996 NRC Canada

Table 6. Evaluation of the absolute (t) and relative (% of annual load) contribution of the spring ice breakup period to the total annual load of particulate and dissolved material carried by the Great Whale River.

	Spring breakup		
	Load	Percentage (%)	Annual value
Discharge (10^9 m^3)			
1986	1.9	10	
1990	2.4	13	190
1991	1.9	10	
PIM (10^3 t)			
1990	30	22	134
1991	15	16	95
POM (10^3 t)			
1986	4.6	na	na
1990	4.8	23	21
1991	5.2	28	19
DOC (10^3 t)			
1990	10	11	90
1991	6.3	7	85
NO_3 (t)	25	12	219
NH_4 (t)	15	5	279
N_{tot} (t)	91	11	864
DRP (t)	9	14	66

Note: The spring ice breakup period is defined as the 15 days following the maximum spring discharge value. na, data not available; all values for 1986 from Hudon (1994); 1990 values = load estimate including the spring 1990 period (June 1990 – May 1991); 1991 values = load estimate including the spring 1991 period (July 1990 – June 1991).

cent nitrogen of CPOM and FPOM (Table 3). Nutrients generated by microbial degradation of POM exhibited a slight winter increase (Table 1), possibly owing to reduced demands by autotrophs (Fig. 4).

Spring ice breakup represented the most dramatic, yet poorly documented event of the hydrological cycle. Warm air temperatures and an increased period of illumination favored rapid melting of the ground snow cover, which generated a rapid increase in river discharge. High surface runoff increased the water level, flooding the river banks and riparian areas that were previously frozen and exposed. River banks were eroded by water, frazil, and ice action, which carried large amounts of PIM and POM into the river. The strong relationships we observed between river discharge and PIM, POM, and DOC likely resulted from this physical entrainment, which took place in the rising part of the hydrograph (Fig. 2). The abundance of POM size fractions was also positively correlated with discharge; at peak spring discharge, CPOM was 25 times less concentrated than FPOM and 500 times less concentrated than VPOM (Fig. 3). Chlorophyll *a* associated with VPOM (i.e., phytoplankton) increased immediately after ice breakup (Fig. 4) resulting from both higher nutrient concentrations via surface runoff (Table 1) and increased light intensity from the removal of the ice cover. In contrast with VPOM, activity associated with larger POM size fractions responded slowly in spring; pigment concentration in CPOM and FPOM (Fig. 4) and microbial assimilation of glutamic acid (Fig. 5) remained near winter levels. Large numbers of insects and fish larvae were swept downstream at this time of the year (Hudon 1994), which also coincided with the congregation of large numbers of juvenile and adult fish in the estuary (Morin et al. 1992).

Contribution of spring ice breakup to yearly load

The period of ice breakup (defined here as the 15 days following the initial peak of spring discharge) is critical to the particulate load of the river. Over this short (5% of the year) period, 10–13% of the annual discharge takes place, coincident with 25–35% of the annual particulate load (Table 6). Concentrations of inorganic and of coarse (>1 mm) organic particles were highly influenced by peak discharge values and thus exhibited high year to year variability.

The total suspended load carried yearly by the Great Whale River was previously estimated at roughly 500 000 t (d'Anglejan and Biksham 1988; from only two measurements); this value is higher than our estimates (94 000 – 135 000 $t \cdot year^{-1}$). The estimate of PIM, POM, and DOC yearly loads are critically influenced by the interpolation model selected. We selected nonlinear regression models between concentration and discharge for their usefulness as predictive tools. These models also provided a reasonably good fit for the sharp increase in concentration with rapidly rising discharge at ice breakup. Accurate modelling of this short-lived period is critical for estimating the maximum spring loads, which, in the case of PIM especially, can represent a significant proportion of the yearly load.

Hudon (1994) estimated that 8.8 t was the load of CPOM (>500 μm) carried to Hudson Bay over the 15 days following the spring 1986 ice breakup. From the results of the present study, this value would roughly translate into a 220-t load of FPOM and a 4400-t load of VPOM. The sum of these three size fractions (4600 t) was in the same order of magnitude as the POM loads measured for the corresponding ice breakup period in June 1990 (4800 t; 23% of yearly 1990 POM load) and May–June 1991 (5200 t; 28% of yearly 1991 POM load).

In contrast with PIM and POM, dissolved substances did not exhibit a steep increase with discharge. The percentage of the total yearly output of DOC and dissolved nutrients at ice breakup was similar to (or lower than, e.g., NH_4) that of water discharge (10–14%; Table 6). The period of ice breakup, therefore, does not contribute to an additional inflow of these components from the Great Whale River.

Great Whale River contributions to coastal Hudson Bay

Nutrient availability (especially nitrogen) was reported to limit biological production in Hudson Bay (see the review of Roff and Legendre 1987). Data from the literature were gathered to compare the concentrations of nutrients and organic carbon we observed in the river with those in Hudson Bay coastal waters (Table 7). River waters (1.0 μM) were less concentrated in NO_3 than Hudson Bay waters (about 2.0 μM), when sampled either under the ice (Legendre et al. 1981; Demers et al. 1989) or in the underlying water column (Gosselin et al. 1985). The spring phytoplankton bloom rapidly depleted the available NO_3 during the open water season (0.5 μM; mid-July to mid-September; Legendre et al. 1982). Fall concentrations for the river and Hudson Bay waters were in the same range (0.2 μM; Legendre and Simard 1979). Also, concentrations of DRP^1 in river waters were consistently lower than those reported in Hudson Bay. These results do not support the hypothesis that fresh waters are a major source of nutrients for coastal waters.

© 1996 NRC Canada

Table 7. Comparison of nutrient, DOC, POC, and chlorophyll *a* (Chl *a*) concentrations observed in the Great Whale River (this study) with those reported for Hudson Bay coastal waters in spring and fall.

	Season	River	Hudson Bay	Reference
NO_3 (μM)	Spring	1.0	2.2	Gosselin et al. 1985
	Fall	0.2	0.19	Legendre and Simard 1979
DRP (μM)	Spring	0.2	0.6	Gosselin et al. 1985
	Fall	0.1	0.42	Legendre and Simard 1979
DOC ($g \cdot m^{-3}$)	Spring	6.0	na	
	Fall	4.5	na	
POC ($g \cdot m^{-3}$)	Spring	1.5	na	
	Fall	1.0	0.10–0.25	Legendre and Simard 1979
			0.05–0.07	Anderson and Roff 1980
Chl *a* ($mg \cdot m^{-3}$)	Spring	0.9	4.0	Michel et al. 1993
	Fall	0.7	0.8	Legendre and Simard 1979

Note: Values for NO_3 and DRP were sampled at 10 m depth. na, data not available.

Increased mixing and physical entrainment of deep, nutrient-rich waters in the coastal zone should be examined as alternative processes explaining the relatively higher productivity of coastal Hudson Bay waters in comparison with the offshore areas (Anderson and Roff 1980).

Chlorophyll *a* concentrations in the river were about four times lower than those reported for the spring phytoplankton bloom in the bay (Michel et al. 1993) but were comparably low (<1 $mg \cdot m^{-3}$) for the remainder of the open water period (Legendre and Simard 1979).

The Great Whale River's major contribution to coastal waters appears to be in the form of POC and DOC (Table 7). POC concentrations exported by the river in spring (0.8 $g \cdot m^{-3}$) and fall (0.5 $g \cdot m^{-3}$) are 2–10 times higher than in Hudson Bay. The contribution of nonrefractory carbon to POC load could be significant: free bacteria in the VPOM fraction (Fig. 5A) contribute 0.2 g $C \cdot m^{-3}$ to the bay (25% of total POC) in the spring. This estimate does not account for bacteria attached to particles; however, free-living bacteria likely represent an important fraction of the total river bacterial population owing to the consistently higher activity measured in the VPOM than in the larger particulate size fractions.

We estimated that about 10^5 t of organic carbon (TOC) was exported yearly from the Great Whale River, 90% of which was DOC (Table 6). These results indicate that the concentrations of organic carbon of continental origin supplied by the Great Whale River could exceed that produced in the marine coastal environment of Hudson Bay. To our knowledge, no comparable values are published for DOC concentrations in Hudson Bay waters. DOC was reported to be limiting to arctic marine bacterial production (Bunch and Harland 1990). However, as with POC, the relative importance of the biologically available versus the refractory fraction (colloidal forms: humic and fulvic acids) must be assessed to evaluate DOC's potential contribution to the marine system (Burton 1988; Parsons et al. 1989; Spitzy and Leenheer 1991).

Comparison of Great Whale River with other large Precambrian Shield rivers

The results of the correlations among watershed characteristics (Table 5) highlight the mutual dependence between climate, precipitation, terrestrial production, and river characteristics; strong correlations were observed between latitude, runoff, and vegetation. The Great Whale River fits the general model between watershed surface area and carbon exports well (Schlesinger and Melack 1981; Mulholland and Watts 1982). Although intense human activities have perceptibly increased nutrient concentrations in many of the rivers flowing into the St. Lawrence and Ottawa rivers (Ministère de l'Environnement du Québec 1993), DOC concentrations in more populated watersheds (e.g., L'Assomption, du Nord, Ottawa) were not higher than in other, less populated ones (e.g., Jacques Cartier). This could result from the small contribution (10% of total) of organic carbon from sewage effluents to the yearly carbon budget of large temperate rivers, which was documented for the populated Hudson River watershed (Gladden et al. 1988).

The emergence of strong relationships between runoff and area-specific DOC load was beyond our expectations, given the error introduced by the use of different methods (Koprivnjak et al. 1994) and the use of a single value to calculate the yearly river DOC exports. The success of the comparison is in part due to the homogeneous geological setting and the large latitudinal and vegetation gradients over which the rivers were selected. It nevertheless points out the importance of watershed characteristics, especially runoff and vegetation, in the determination of DOC concentrations in Precambrian Shield rivers.

In agreement with our findings, Engstrom (1987) showed the influence of vegetation and hydrology on the humus budgets of Labrador lakes, along a gradient ranging from clear northern tundra lakes to tea-colored, more southern boreal forest lakes. In addition to differences in vegetation, forested and tundra watersheds differ markedly in the rate of production and degradation (hence in standing stock) of terrestrial carbon and in the contribution of groundwater flow to river DOC, which is likely reduced in areas subjected to permafrost. In contrast, wetlands (Eckhardt and Moore 1990) and swamp-draining streams (Mulholland and Kuenzler 1979) were documented to have a higher rate of DOC exports than the upland rivers studied here. A high proportion of wetlands in the Matamek river catchment could explain the high DOC concentrations reported in two of the three studies of this pristine watershed (outliers in Fig. 6; Naiman 1982; Walsh et al. 1987). Both values were excluded from the linear regression between DOC export and runoff to obtain a conservative estimate of the dif-

© 1996 NRC Canada

ference between groups of rivers draining into the Gulf of St. Lawrence and Hudson–Ungava bay basins.

The linear regressions we calculated are very similar to the equation obtained from Spitzy and Leenheer's (1991) data for large rivers of the world (DOC exports = 0.57 + 0.004(runoff), $n = 20$, $r^2 = 0.51$), and to Mulholland and Kuenzler's (1979) equation (DOC exports = 0.34 + 0.002(runoff), $n = 10$, $r^2 = 0.91$), for a variety of small upland rivers. Such relationships are invaluable for quantitative estimates of terrestrial carbon transport to the world's oceans and support the predominant influence of runoff across a wide range of river sizes and characteristics. Large rivers are important contributors of terrestrial allochtonous matter to coastal zones around the world, and our results indicate that the Great Whale River is no exception.

Acknowledgements

Edward Mamianskum provided field assistance and sampling. The help of the personnel of the Centre d'études nordiques in Kuujjuarapik (Université Laval), and particularly of Charles Côté, was greatly appreciated. Glynnis de Sylveira (Pulp and Paper Research Institute of Canada) made the scanning electron microscopic observations and took micrographs. Luzette Teruel and Ben Simpson (Macdonald Campus of McGill University) provided the Kjeldahl analyses for particulate size fractions. Ray Hesslein (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man.) looked after the C:N analyses. Gordon Walsh and Michel Gilbert (Department of Fisheries and Oceans, Institut Maurice Lamontagne, Mont-Joli, Que.) financed part of the project. We thank Aline Sylvestre (Centre Saint-Laurent), who generously provided unpublished data for northern river basins. The suggestions of Richard Carignan and of two anonymous referees are gratefully acknowledged.

References

American Public Health Association. 1981. Standard methods for the examination of water and wastewater. 15th ed. American Public Health Association, American Water Works Association, amd Water Pollution Control Federation, Washington, D.C.

Anderson, J.T., and Roff, J.C. 1980. Seston ecology of the surface waters of Hudson Bay. Can. J. Fish. Aquat. Sci. **37**: 2242–2253.

Bilby, R.E., and Likens, G.E. 1980. Importance of organic debris dams in the structure and function of stream ecosystems. Ecology, **61**: 1107–1113.

Boling, R.H., Goodman, E.D., Van Sickle, J.A., Zimmer, J.O., Cummings, K.W., Petersen, R.C., and Reice, S.R. 1975. Toward a model of detritus processing in a woodland stream. Ecology, **56**: 141–151.

Bratbak, G., and Dundas, I. 1984. Bacterial dry matter content and biomass estimations. Appl. Environ. Microbiol. **48**: 755–757.

Bunch, J.N. 1987. Effects of petroleum releases on bacterial numbers and microheterotrophic activity in the water and sediment of an arctic marine ecosystem. Arctic, **40**: 172–183.

Bunch, J.N., and Harland, R.C. 1990. Bacterial production in the bottom surface of sea ice in the Canadian subarctic. Can. J. Fish. Aquat. Sci. **47**: 1986–1995.

Bunch, J.N., and Reeves, R.R. (*Editors*). 1992. Proceedings of a Workshop on the Potential Cumulative Impacts of Development in the Region of Hudson and James Bays, 17–19 June 1992, Ottawa, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. No. 1874.

Burton, J.D. (*Editor*). 1988. River inputs to oceans systems: status and recommendations for research. Final report of SCOR Working Group 46. UNESCO Tech. Pap. Mar. Sci. No. 55.

Campbell, P.G.C., Hansen, H.J., Dubreuil, B., and Nelson, W.O. 1992. Geochemistry of Quebec North Shore salmon rivers during snowmelt: organic acid pulse and aluminum mobilization. Can. J. Fish. Aquat. Sci. **49**: 1938–1952.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Annu. Rev. Entomol. **18**: 183–206.

d'Anglejan, B., and Biksham, G. 1988. Late winter – early spring sedimentation off the Great Whale River, southeastern Hudson Bay. Can. J. Earth Sci. **25**: 930–933.

Degens, E.T., Kempe, S., and Richey, J.E. 1991. Biogeochemistry of major world rivers. *In* SCOPE No. 42. Wiley, New York.

Demers, S., Legendre, L., Maestrini, S.Y., Rochet, M., and Ingram, R.G. 1989. Nitrogenous nutrition of sea-ice microalgae. Polar Biol. **9**: 377–383.

Dodge, D.P. (*Editor*). 1989. Proceedings of the International Large River Symposium (LARS), Honey Harbour, Ont., 14–21 September 1986. Can. Spec. Publ. Fish. Aquat. Sci. No. 106.

Eckhardt, B.W., and Moore, T.R. 1990. Controls on dissolved organic carbon concentrations in streams, southern Quebec. Can. J. Fish. Aquat. Sci. **47**: 1537–1544.

Engstrom, D. 1987. Influence of vegetation and hydrology on the humus budgets of Labrador lakes. Can. J. Fish. Aquat. Sci. **44**: 1306–1314.

Environment Canada. 1992. Historical streamflow summary to 1990. Inland Waters Directorate, Quebec Region, Water Quantity and Quality Branch, Longueuil, Que.

Fisher, S.G. 1977. Organic matter processing by a stream ecosystem: Fort River, Massachusetts, U.S.A. Int. Rev. Gesamten Hydrobiol. **62**: 701–727.

Fisher, S.G., and Likens, G.E. 1973. Energy flows in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol. Monogr. **43**: 421–439.

Gladden, J.B., Cantelmo, F.C., Croom, J.M., and Shapot, R. 1988. An evaluation of the Hudson River ecosystem in relation to the dynamics of fish populations. *In* Science, law, and Hudson River power plants: a case study in environmental impact assessment. *Edited by* L.W. Barnhouse, F.J. Klauda, D.S. Vaughan, and R.L. Kendall. Am. Fish. Soc. Monogr. **4**: 37–52.

Gosselin, M., Legendre, L., Demers, S., and Ingram, R.G. 1985. Responses of sea-ice microalgae to climatic and fortnightly tidal energy inputs (Manitounuk Sound, Hudson Bay). Can. J. Fish. Aquat. Sci. **42**: 999–1006.

Hudon, C. 1994. Biological events during ice breakup in the Great Whale River (Hudson Bay). Can. J. Fish. Aquat. Sci. **51**: 2467–2481.

Jackson, T.A. 1975. Humic matter in natural waters and sediments. Soil Sci. **119**: 56–64.

Jeffrey, S.W., and Humphrey, G.F. 1975. New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. **167**: 191–194.

Koprivnjak, J.-F., Blanchette, J.G., Bourbonnière, R.A., Clair, T.A., Heyes, A., Lum, K.R., McCrea, R., and Moore, T.R. 1994. The underestimation of concentrations of dissolved organic carbon in freshwaters. Water Res. **29**: 91–94.

Legendre, L., Ingram, R.G., and Poulin, M. 1981. Physical control of phytoplankton production under sea ice (Manitounuk Sound, Hudson Bay). Can. J. Fish. Aquat. Sci. **38**: 1385–1392.

Legendre, L., Ingram, R.G., and Simard, Y. 1982. Aperiodic changes of water column stability and phytoplankton in an arctic coastal embayment, Manitounuk Sound, Hudson Bay. Nat. Can. (Que.), **109**: 775–786.

Legendre, L., and Simard, Y. 1979. Océanographie biologique estivale et phytoplancton dans le sud-est de la Baie d'Hudson. Mar. Biol. (Berlin), **52**: 11–22.

© 1996 NRC Canada

Michel, C., Legendre, L., Therriault, J.-C., Demers, S., and Vandevelde, T. 1993. Springtime coupling between ice algal and phytoplankton assemblages in southeastern Hudson Bay, Canadian Arctic. Polar Biol. **13**: 441–449.

Ministère de l'Environnement du Québec. 1991. Annuaire hydrologique. 1990–1991. Ministère de l'Environnement du Québec, Direction du réseau hydrique, Québec, Que.

Ministère de l'Environnement du Québec. 1993. État de l'environnement au Québec, 1992. Guérin, Montréal, Que.

Ministère des Forêts du Québec. 1991. Les zones de végétation au Québec. Ministère des Forêts du Québec, Québec, Que.

Ministère des Richesses Naturelles du Québec. 1969. Superficies des bassins versants du Québec. Ministère des Richesses Naturelles du Québec, Service de l'hydrographie, Québec, Que.

Morin, B., Hudon, C., and Whoriskey, F.G. 1992. Environmental influences on seasonal distribution of coastal and estuarine fish assemblages at Wemindji, eastern James Bay. Environ. Biol. Fishes, **35**: 219–229.

Mulholland, P.J., and Kuenzler, E.J. 1979. Organic carbon from upland and forested wetlands watersheds. Limnol. Oceanogr. **24**: 960–966.

Mulholland, P.J., and Watts, J.A. 1982. Transport of organic carbon to the oceans by rivers of North America: a synthesis of existing data. Tellus, **34**: 176–186.

Naiman, R.J. 1982. Characteristics of sediment and organic carbon export from pristine boreal forest watersheds. Can. J. Fish. Aquat. Sci. **39**: 1699–1718.

Parsons, T.R., Webb, D.G., Rokeby, B.E., Lawrence, M., Hopky, G.E., and Chiperzak, D.B. 1989. Autotrophic and heterotrophic production in the Mackenzie River/Beaufort Sea estuary. Polar Biol. **9**: 261–266.

Petersen, R.C., Jr., Cummins, K.W., and Ward, G.M. 1989. Microbial and animal processing of detritus in a woodland stream. Ecol. Monogr. **59**: 21–39.

Pocklington, R., and Tan, F. 1987. Seasonal and annual variations in the organic matter contributed by the St. Lawrence River to the Gulf of St. Lawrence. Geochim. Cosmochim. Acta, **51**: 2579–2586.

Richard, Y., Laflamme, N., and Moreau, G. 1982. Colonisation par les micro-organismes, évolution chimique des feuilles de différentes espèces d'arbres (peuplier, aulne, myrique) dans les eaux oligotrophes du Bouclier canadien et incidence sur leur utilisation par les macro-invertébrés. Hydrobiologia, **96**: 65–75.

Roff, J.C., and Legendre, L. 1987. Physico-chemical and biological oceanography. *In* Canadian inland seas. *Edited by* I.P. Martini. Elsevier Oceanogr. Ser. No. 44. pp. 265–291.

Schlesinger, W.H., and Melack, J.M. 1981. Transport of organic carbon in the world's rivers. Tellus, **33**: 172–187.

Société d'énergie de la Baie James. 1978. Connaissance du milieu des territoires de la Baie James et du Nouveau Québec. Société d'énergie de la Baie James, Montréal, Que.

Spitzy, A., and Leenheer, J. 1991. Dissolved organic carbon in rivers. *In* Biogeochemistry of major world rivers. *Edited by* E.T. Degens, S. Kempe, and J.E. Richey. *In* SCOPE No. 42. Wiley, New York. pp. 212–232.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of fresh water. 2nd ed. Fish. Mar. Serv. Misc. Publ. No. 25. Department of Fisheries and Oceans, Winnipeg, Man.

Strickland, J.D.H., and Parsons, T.R. 1972. A practical handbook of seawater analysis. 2nd ed. Bull. Fish. Res. Board Can. No. 167.

Technicon Industrial Systems. 1973. Orthophosphate in water and sea water. Industrial method No. 155–71W. Technicon Industrial Systems, Tarrytown, N.Y.

U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. U.S. Environ. Prot. Agency Res. Dev. Rep. No. EPA–600/4–79–020. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. pp. 353.1–1 – 353.1–3.

Walsh, G., and Vigneault, Y. 1986. Analyse de la qualité de l'eau de rivières de la Côte-Nord du Golfe St-Laurent en relation avec les processus d'acidification. Can. Tech. Rep. Fish. Aquat. Sci. No. 1540.

Walsh, G., Verreault, G., and Vigneault, Y. 1987. Acidification minérale et organique des rivières de la Côte-Nord (Golfe du Saint-Laurent). Nat. Can. (Rev. Écol. Syst.) **114**: 262–282.

Watson, S.W., Novitsky, T.V., Quinby, H.L., and Valois, F.W. 1977. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. **33**: 940–946.

Wetzel, R.G., and Likens, G.E. 1991. Limnological analyses. 2nd ed. Springer-Verlag, New York.

Appendix. Summary of watershed characteristics for the 47 rivers used in the DOC model.

No.	River	Basin	Watershed (km^2)	Discharge ($m^3 \cdot s^{-1}$)	Runoff ($mm \cdot year^{-1}$)	Lat.	Veg.	Dissolved Organic Carbon: Conc. ($mg \cdot L^{-1}$)	Load ($t \cdot year^{-1}$)	Sp. load ($t \cdot km^{-2} \cdot year^{-1}$)	Reference
1	Great Whale	SHB	42 735	666	491	55.17	Tai	4.25	89 263	2.09	This study
1.1	Great Whale	SHB	42 735	666	491	55.17	Tai	3.35	70 360	1.65	A. Sylvestre[a]
2	Little Whale	SHB	15 850	167	332	55.44	FoT	3.05	16 063	1.01	A. Sylvestre[a]
3	du Nord	SHB	1 437	37	812	56.32	FoT	3.30	3 851	2.68	A. Sylvestre[a]
4	Nastapoka	SHB	13 364	256	604	56.51	FoT	3.67	29 629	2.22	A. Sylvestre[a]
5	Arnaud (Payne)	UB	49 469	636	405	59.59	ArT	1.37	27 478	0.56	A. Sylvestre[a]
6	aux Feuilles	UB	42 476	602	447	58.38	ArT	1.65	31 325	0.74	A. Sylvestre[a]
7	aux Melezes	UB	42 735	620	458	57.4	FoT	2.76	53 964	1.26	A. Sylvestre[a]
8	Caniapiscau	UB	89 610	1 713	603	57.25	FoT	2.75	148 558	1.66	A. Sylvestre[a]
9	Whale	UB	31 857	555	549	57.53	FoT	3.07	53 733	1.69	A. Sylvestre[a]
10	Georges	UB	41 699	877	663	58.09	FoT	1.93	53 378	1.28	A. Sylvestre[a]
11	Saint-Augustin	GSL	9 894	306	976	51.36	Bor	5.90	56 988	5.76	Walsh and Vigneault 1986
12	Gros Mecatina	GSL	992	24	775	50.25	Bor	5.30	4 074	4.11	Walsh and Vigneault 1986
13	Petit Mecatina	GSL	19 580	347	558	50.24	Bor	5.90	64 472	3.29	Walsh and Vigneault 1986
14	Etamamiou	GSL	3 030	105	1090	50.23	Bor	4.30	14 207	4.69	Walsh and Vigneault 1986
15	Olomane	GSL	5 439	163	946	50.23	Bor	5.70	29 331	5.39	Walsh and Vigneault 1986
16	Musquaro	GSL	3 626	77	672	50.20	Bor	4.20	10 232	2.82	Walsh et al. 1987

© 1996 NRC Canada

Appendix (*concluded*).

No.	River	Basin	Watershed (km^2)	Discharge ($m^3 \cdot s^{-1}$)	Runoff ($mm \cdot year^{-1}$)	Lat.	Veg.	Dissolved Organic Carbon: Conc. ($mg \cdot L^{-1}$)	Dissolved Organic Carbon: Load ($t \cdot year^{-1}$)	Dissolved Organic Carbon: Sp. load ($t \cdot km^{-2} \cdot year^{-1}$)	Reference
16.1	Musquaro	GSL	3 626	77	672	50.20	Bor	5.70	13 886	3.83	
17	Natashquan	GSL	16 110	422	826	50.11	Bor	5.10	67 853	4.21	Walsh and Vigneault 1986
18	Watshishou	GSL	1 052	26	779	50.15	Bor	4.60	3 772	3.59	Walsh et al. 1987
19	Corneille	GSL	559	15	825	50.20	Bor	4.40	2 030	3.63	Walsh and Vigneault 1986
20	Romaine	GSL	14 349	338	742	50.18	Bor	4.60	48 996	3.41	Walsh and Vigneault 1986
21	Magpie	GSL	7 641	180	742	50.19	Bor	3.90	22 105	2.89	Walsh and Vigneault 1986
22	Tonnerre	GSL	694	21	945	50.16	Bor	4.30	2 821	4.06	Walsh and Vigneault 1986
23	Matamek	GSL	684	19	876	50.17	Bor	6.90	4 134	6.04	Walsh and Vigneault 1986
23.1	Matamek	GSL	684	19	876	50.17	Bor	8.40	5 033	7.36	Walsh et al. 1987
23.2	Matamek	GSL	684	19	876	50.17	Bor	11.30	6 771	9.90	Naiman 1982
24	Moisie	GSL	19 191	466	766	50.21	Bor	4.40	64 661	3.37	Walsh and Vigneault 1986
24.1	Moisie	GSL	19 191	466	766	50.21	Bor	6.80	99 931	5.21	Naiman 1982
25	aux Rochers	GSL	4 170	87	658	50.05	Bor	7.00	19 205	4.61	Campbell et al. 1992
26	MacDonald	GSL	682	17	786	50.05	Bor	7.60	4 074	5.97	Campbell et al. 1992
27	Trinite	GSL	562	15	842	49.24	Bor	5.70	2 696	4.80	Campbell et al. 1992
27.1	Trinite	GSL	562	15	825	49.24	Bor	5.20	2 410	4.29	Walsh and Vigneault 1986
28	Godbout	GSL	1 575	43	862	49.19	Bor	5.60	7 600	4.83	Walsh and Vigneault 1986
29	Manicouagan	GSL	45 843	1 006	692	49.12	Bor	4.50	142 763	3.11	Walsh and Vigneault 1986
29.1	Manicouagan	GSL	45 843	1 006	692	49.12	Bor	4.00	126 901	2.77	MENVIQ 1993[b]
30	Aux Outardes	GSL	19 069	418	691	49.09	Bor	4.80	63 274	3.32	Walsh and Vigneault 1986
30.1	Aux Outardes	GSL	19 069	418	691	49.09	Bor	4.40	58 001	3.04	MENVIQ 1993[b]
31	Bersimis	GSL	18 700	333	561	49.00	Bor	5.20	54 544	2.92	Walsh and Vigneault 1986
32	des Escoumins	GSL	798	16	632	48.20	Bor	4.50	2 271	2.85	Walsh and Vigneault 1986
33	Saguenay	GSL	88 060	1 760	630	48.26	Mix	6.15	341 346	3.88	MENVIQ 1993[b]
34	Sainte-Marguerite	GSL	2 132	59	873	48.16	Bor	3.90	7 256	3.40	Walsh and Vigneault 1986
35	Saint-Charles	SLR	507	15	933	46.6	Mix	3.80	1 798	3.55	MENVIQ 1993[b]
36	Jacques-Cartier	SLR	2 515	77	966	46.53	Bor	4.60	11 170	4.44	MENVIQ 1993[b]
37	Sainte-Anne	SLR	2 662	80	948	46.3	Dec	4.00	10 092	3.79	MENVIQ 1993[b]
38	Saint-Maurice	SLR	43 253	704	513	46.26	Mix	6.50	144 309	3.34	MENVIQ 1993[b]
39	du Loup	SLR	1 510	35	731	46.15	Dec	4.60	5 077	3.36	MENVIQ 1993[b]
40	Maskinonge	SLR	1 126	27	756	46.1	Dec	5.20	4 428	3.93	MENVIQ 1993[b]
41	Assomption	SLR	4 173	79	597	46	Dec	4.80	11 958	2.87	MENVIQ 1993[b]
42	Ottawa	OR	143 000	1 940	428	45.34	Dec	5.90	360 961	2.52	MENVIQ 1993[b]
43	du Nord	OR	2 189	40	576	45.5	Dec	4.60	5 803	2.65	MENVIQ 1993[b]
44	Rouge	OR	5 460	105	606	45.44	Dec	4.35	14 404	2.64	MENVIQ 1993[b]
45	du Lievre	OR	9 472	176	586	45.34	Dec	4.50	24 977	2.64	MENVIQ 1993[b]
46	Gatineau	OR	23 724	355	472	45.29	Dec	5.00	55 976	2.36	MENVIQ 1993[b]
47	St.Lawrence	SLR	1 424 000	12 639	280	46.5	Dec	4.90	1 953 059	1.37	Pocklington and Tan 1987

Note: Hydrographic basins of southeastern Hudson (SHB) and Ungava bays (UB), Gulf of St.Lawrence (GSL), and Ottawa (OR) and St.Lawrence rivers (SLR) are identified. Vegetation types (Veg.) refer to deciduous (Dec), mixed (Mix), and boreal (Bor) forests, and taiga (Tai), forested (FoT), and arctic (ArT) tundra (Ministère des Forêts du Québec 1991). Yearly annual discharge (YAD) of Watshishou, aux Rochers, and MacDonald rivers was calculated using the relation log YAD ($m^3 \cdot s^{-1}$) = −1.28 + 0.89 log watershed surface area (km^2). Lat., latitude.

[a]Personal communication, Centre Saint-Laurent, 105 McGill street, Montreal, QC H2Y 2E7, Canada.

[b]Ministère de l'Environnement du Québec.

© 1996 NRC Canada

Congener-specific exposure of grey seal (*Halichoerus grypus*) pups to chlorinated biphenyls during lactation

P.P. Pomeroy, N. Green, A.J. Hall, M. Walton, K. Jones, and J. Harwood

Abstract: We determined total and daily exposure of young grey seals (*Halichoerus grypus*) to 25 chlorinated biphenyl (CB) congeners in their mothers' milk at the Isle of May, Scotland, in 1990, 1991, and 1992. Total exposure for 36 pups born to 13 different females varied between 8.8 and 58.9 mg; IUPAC congeners CB-153 and CB-138 were the dominant components in milk, accounting for 42.1% of total CB exposure. The quantity of milk consumed by pups each day and the lipid content of that milk increased during lactation. As a result, daily exposure to CBs increased sharply in the first half of the 18-day lactation period. There were significant among-female differences in milk CB concentrations and in the relative contribution of individual congeners to total CB exposure. When the effects of among-female variation were removed, there were significant changes in concentrations of less chlorinated CBs during lactation and among years. A grey seal pup's exposure to CBs can be accurately estimated from its mother's identity, its mass at weaning, and the CB concentration in a single milk sample taken towards the end of lactation.

Résumé : Nous avons mesuré l'exposition totale et quotidienne de jeunes phoques gris (*Halichoerus grypus*) à 25 congénères de biphényle chloré (BC) présents dans le lait des mères à l'île de May (Écosse) en 1990, 1991 et 1992. L'exposition totale de 36 petits nés de 13 femelles différentes variait entre 8,8 et 58,9 mg; les congénères UICPA CB-153 et CB-138 étaient les composantes principales dans le lait, où ils représentaient 42,1% de l'exposition totale aux BC. La quantité de lait consommée chaque jour par les petits et la teneur en lipides de ce lait augmentaient pendant la lactation. En conséquence, l'exposition quotidienne aux BC a augmenté fortement pendant la première moitié de la période d'allaitement de 18 jours. Il y avait des différences significatives entre les femelles dans les concentrations de BC dans le lait et dans la contribution relative des différents congénères à l'exposition totale aux BC. Lorsqu'on éliminait les effets des variations entre femelles, on observait des changements significatifs dans les concentrations de BC moins chlorés pendant la lactation et d'une année à l'autre. On peut estimer de façon précise l'exposition d'un petit phoque gris aux BC si l'on connaît l'identité de sa mère, sa masse au moment du sevrage et la concentration de BC dans un échantillon de lait prélevé vers la fin de l'allaitement.
[Traduit par la Rédaction]

Introduction

Chlorinated biphenyls (CBs) are persistent man-made compounds whose presence has been associated with reproductive disorders (Helle et al. 1976; Reijnders 1986) and immune system dysfunction (de Swart et al. 1994; Ross et al. 1993) in seals. A total of 209 separate CB isomers are possible, but in practice, fewer than 100 congeners appear in mixtures entering the environment (Duinker et al. 1988).

During their short lactation period, phocid seals mobilize relatively large quantities of stored fat reserves to feed their offspring. In the United Kingdom, female grey seals (*Halichoerus grypus*) sustain all of their energy requirements during lactation from reserves brought ashore before the birth of the pup. Grey seal pups receive milk that contains up to 60% lipid by mass for approximately 18 days (Baker 1990; Iverson et al. 1993). Highly lipophilic organochlorine compounds, such as CBs, that have accumulated in the blubber are mobilized by females as milk is produced. As a result, a significant part of a female's contaminant burden is delivered to her pup at a time when the pup's immune system is still developing and may be particularly sensitive to the effects of these compounds (Carter et al. 1990; Ross et al. 1993).

Previous studies of CB levels in phocid milk have reported levels in single samples from a few individuals (Schweigert and Knöppler 1990; Bacon et al. 1992), or they have used samples collected from dead animals at different stages of lactation to estimate body burdens and total quantities of CB mobilized by females (Addison and Brodie 1977, 1987). In some cases, estimates have been quantified on the basis of the composition of standard commercial mixtures of CBs. This ignores the effects of differential uptake, bioaccumulation, metabolism, and mobilization of the different congeners (Addison and Brodie 1977; Boon and Eijgenraam 1988).

In this paper we estimate the quantities of individual CB congeners transferred from individually marked grey seal mothers to their offspring in 3 consecutive years at the Isle of May, Scotland. CB concentrations were measured in milk samples collected from each female on a number of occasions during lactation. The total quantity of milk and milk fat

Received June 15, 1995. Accepted January 29, 1996.
J12956

P.P. Pomeroy,[1] A.J. Hall, M. Walton, and J. Harwood. Natural Environment Research Council Sea Mammal Research Unit, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, FIFE KY16 8LB, U.K.
N. Green and K. Jones. Department of Biological and Environmental Sciences, University of Lancaster, Lancaster, L1A 4YQ, U.K.

[1] Author to whom all correspondence should be addressed. e-mail: pppo.wpo.nerc.ac.uk

© 1996 NRC Canada

Table 1. Details of grey seal mothers and pups studied at the Isle of May.

Mother	Age in 1990	Year	Lactation duration (days)	Pup weaning mass (kg)	Pup growth rate ($kg \cdot day^{-1}$)	Total milk intake (kg)	Total milk lipid (kg)	Pup sex	Total CB exposure (mg)
0W	7	1990	17	24.6	0.58	31.65	14.20	M	16.84
		1991	14	43.8	1.64	41.07	18.96	M	15.27
1W	6	1990	13	27.3	0.82	36.80	19.97	M	22.89
		1991	4	14.2	−0.33	1.26	0.60	M	0.96
		1992	18	42.5	1.52	53.45	20.59	F	37.47
2B	6	1990	15	39.5	1.62	46.59	20.63	F	24.84
		1991	21	45.5	1.43	59.95	27.86	M	37.73
		1992	21	52.0	1.74	68.53	32.38	M	31.78
2W	13	1990	13	33.1	1.13	43.47	12.48	M	50.33
		1992	15	43.8	1.89	51.46	21.81	M	58.85
7B	6	1990	15	24.0	0.71	29.72	16.22	F	21.31
		1991	18	36.3	1.17	46.18	20.92	F	16.92
		1992	17	41.7	1.54	49.91	22.87	M	26.48
9B	5	1990	17	23.8	0.68	33.64	19.68	M	16.46
		1991	17	21.4	0.37	27.35	10.87	M	8.79
		1992	19	53.4	1.80	62.83	34.11	F	34.34
9H	9	1991	16	36.9	1.24	41.27	16.08	M	26.78
		1992	17	32.0	0.92	38.69	15.85	M	20.10
D0	13	1990	18	33.2	1.00	41.60	18.63	F	30.22
		1991	16	45.5	1.76	50.62	19.76	M	28.57
		1992	15	38.7	1.57	46.48	19.10	F	44.42
D1	15	1990	21	50.7	1.69	66.66	31.46	F	31.26
		1991	21	49.5	1.58	62.17	27.77	M	31.50
		1992	18	49.0	1.88	59.27	27.38	M	29.26
D6	10	1990	17	35.6	1.18	44.46	19.64	M	17.81
		1991	17	41.2	1.41	46.99	19.79	M	19.84
		1992	16	39.4	1.49	47.35	22.65	M	18.77
D8	?	1990	16	38.4	1.43	46.43	20.48	M	20.37
		1991	16	45.9	1.92	55.29	26.46	F	33.31
		1992	18	43.4	1.51	51.88	22.80	F	38.19
H7	18	1990	21	53.4	1.76	67.61	31.13	F	26.89
		1991	19	45.8	1.48	55.60	25.38	F	19.32
		1992	20	50.2	1.68	62.89	28.39	M	26.32
X+	22	1990	20	46.4	1.55	60.14	26.40	M	39.62
		1991	20	41.0	1.29	54.15	22.16	F	24.96
		1992	17	39.5	1.43	50.45	22.33	F	23.95

delivered by each female to her pup was estimated using isotope dilution techniques. We also evaluated whether less intensive study methods could be used to estimate pups' exposure to CBs.

Methods

Study population and sample collection

Study animals were chosen from a population of approximately 50 females, individually marked with letter–number ciphers, that breed at the Isle of May (56°12′N, 2°32′W). All females and pups in the study were free ranging; most females remained with or near their pups throughout the lactation period. The breeding areas on the island were observed each day so that we were able to record the date of birth of every pup and the day on which they were weaned. In a few cases, pups were born before we arrived on the island; their dates of birth were estimated using the age-related characteristics identified by Kovacs and Lavigne (1986). The results presented here are from the 13 females for which we have the most complete data over the 1990, 1991, and 1992 breeding seasons.

Milk samples were collected during the 18-day lactation period at around days 2, 9, and 16 postpartum. Females were anaesthetized using a pressurized dart to deliver intramuscular injections of Zoletil 100 (Virbac UK Ltd.) at a dose of 1.0 mL/100 kg estimated female mass (Baker et al. 1990). They were weighed to the nearest kilogram at each capture date using a net slung between poles, in line with a load cell, suspended from the apex of a tripod. Pups were secured in canvas bags and weighed to the nearest 0.2 kg on Salter spring scales (Todd Scales, Newmarket, U.K.). Pups were marked with permanent dyes for recognition, tagged in the hind flipper (Rototag, Dalton, U.K.), and replaced with their mothers as soon as they had been sampled.

Milk letdown was stimulated by a 1-mL intravenous injection of oxytocin (Veterinary Drug Co., Bury St. Edmunds, U.K.). The teat area was cleaned with dilute chlorhexidine solution and approximately 60 mL of milk was withdrawn using a clean cut-off syringe. Milk was then transferred to labelled hexane-washed glass pots with lids lined with aluminium foil. Milk samples were frozen at −20°C on the day of collection and remained frozen until analysis.

Milk lipid determination

Thawed milk samples equilibrated to room temperature were

© 1996 NRC Canada

Fig. 1. Changes in the lipid content, as percent hexane extractable lipid, of grey seal milk during the course of lactation.

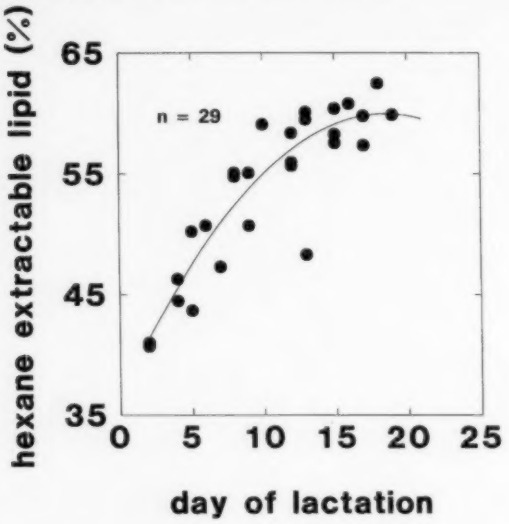
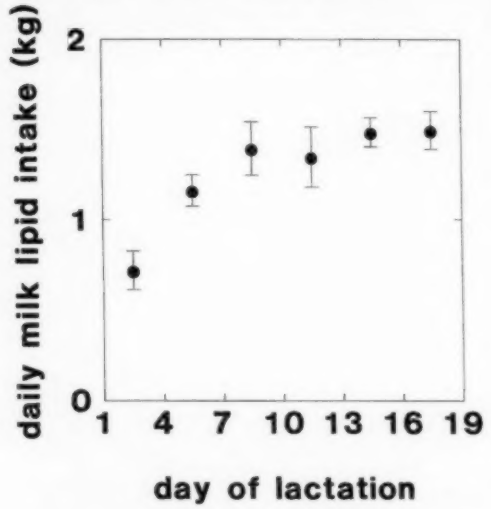


Fig. 2. Changes in the quantity of lipid delivered by grey seal mothers to their pups during the course of lactation.



homogenized by shaking or stirring. Preweighed aliquots of milk samples were added to anhydrous sodium sulphate. Aliquots were spiked with standard amounts of three CB congeners (IUPAC numbers CB-40, CB-155, and CB-185; Ballschmitter and Zell 1980) not found in natural samples. Each aliquot was transferred to a precleaned Buchi 810 soxhlet apparatus and extracted with hexane for 16 h. The extract was concentrated to a volume of approximately 10 mL.

Half of the extract was transferred to a preweighed vial and used to determine lipid content. The solvent was evaporated under a stream of dry nitrogen with the vial maintained at 35°C. The lipid content was calculated by difference. Water, protein, carbohydrate, and ash content of our milk samples were calculated using published values for grey seal milk corresponding to our lipid contents (Iverson et al. 1993).

CB separation and quantification

The remaining half of the extract was transferred with two hexane rinses to a 100-mL separating funnel where the biochemical content (including lipids) and chemically labile organic molecules of the extract were removed by sequential acid digestion (20 mL concentrated sulphuric acid overnight, 10 mL for 4 h, 10 mL for 3 h). The hexane layer was retained at each stage and concentrated to approximately 1 mL in a vial maintained at 35°C under a stream of dry nitrogen. CBs were separated from other organochlorines by adsorption chromatography. A 300 × 7 mm internal diameter column was packed from bottom to top with a plug of glass wool, 2.5 g silica (Merck silica gel 60 (70–230 mesh), activated at 600°C for 8 h then deactivated with 5% water), 3.0 g alumina (BDH neutral Brockman grade 1, activated at 800°C for 12 h then deactivated with 5% water), and anhydrous sodium sulphate (BDH, activated at 450°C for 8 h and stored in a dessicator). The sample was applied to the dry column along with three 0.5- to 1.0-mL rinses and eluted with hexane. The first 9 mL of eluate were collected. Congener 209 was added as an internal retention standard. One millilitre of dodecane was added as a holding solvent, and the hexane was evaporated by maintaining at 35°C under a stream of dry nitrogen. The final extract in dodecane was transferred to a 2-mL vial prebaked at 450°C for 8 h.

Twenty-five CB congeners (IUPAC numbers CB-28, CB-44, CB-52, CBs–61/74, CB-66, CBs–82/151, CB-101, CB-105, CB-110/77, CB-118, CB-128, CB-138, CB-149, CB-153, CB-170, CB-180, CB-183, CB-187, CB-189, CBs–194/205, CB-198, CB-201, CB-202, CB-206, CB-208) were isolated using a Hewlett Packard 5890 series II gas chromatograph (GC) equipped with a HP 7673 autosampler set up to inject a 1-μL sample onto a 50-m Ultra 2 capillary column. Operating conditions for the GC were as follows: injector port temperature 320°C; detector temperature 380°C; oven temperature programme 100°C held for 2 min, ramped at 20°C·min^{-1} to 140°C, ramped 4°C·min^{-1} to 200°C, maintained for 13 min, ramped at 4°C·min^{-1} to 280°C, and held for 8 min. Detection was by electron capture and all data were recorded using Minichrom software. Quantification was by comparison with external standards, using a linear calibration curve and correcting for recoveries on the basis of the three surrogate standards. Tetrachlorinated congeners were corrected using CB-40 recovery; penta- and hexa-chlorinated congeners were corrected using CB-155 recovery and more highly chlorinated congeners were corrected using CB-185 recovery (CB-40: 96 ± 12% recovery (mean ± SD); CB-155: 94 ± 10% recovery; CB-185: 84 ± 12% recovery).

Quality assurance of chemical analyses

A blank was run with each batch of samples processed, and duplicate analyses were run on approximately 10% of samples. Repeated analyses of a single sample were carried out throughout the study. The SDs of these repeated measures were less than 20% of the mean for CB concentrations greater than 5 ng·g^{-1} lipid.

Measurement of body composition and milk intake

Total body composition and water turnover were estimated from isotope dilution techniques, and these values were used to calculate milk intake, following the method described in Oftedal et al. (1987). For females, a blood sample was obtained from the extradural vein and a preweighed dose of tritiated water (approximately 2.0×10^8 Bq in the first instance, 0.7×10^8 Bq at subsequent samplings) was injected directly into the blood. The syringe was rinsed three times with fresh blood. Body composition of pups was determined by injecting a preweighed dose of 99.8% pure deuterium oxide (Aldrich Chemical Co., U.K.) into the intradermal space or extradural vein. Syringes were weighed after injections to determine the mass of deuterium delivered. The total body water pool of pups was assumed to include any milk already ingested at the time of isotope administration. Mothers

© 1996 NRC Canada

Fig. 3. Relative concentrations of individual chlorinated biphenyl (CB) congeners in the milk of grey seals. Concentrations (calculated on a lipid weight basis) are expressed as a proportion of total assayed CB content.

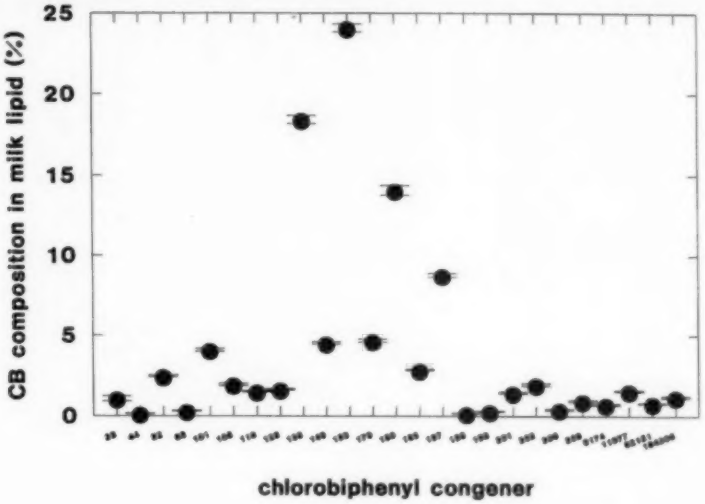


Table 2. Spearman correlations between concentrations of the eight most abundant individual CBs and the sum of assayed CBs in grey seal milk.

Congener	CB-101	CB-138	CB-149	CB-153	CB-170	CB-180	CB-183	CB-187	Total CB
CB-101	1.000	0.626	0.831	0.551	0.521	0.530	0.548	0.676	0.622
CB-138		1.000	0.776	0.927	0.692	0.667	0.704	0.733	0.811
CB-149			1.000	0.764	0.699	0.678	0.744	0.791	0.800
CB-153				1.000	0.833	0.787	0.834	0.806	0.867
CB-170					1.000	0.916	0.986	0.883	0.845
CB-180						1.000	0.923	0.858	0.817
CB-183							1.000	0.902	0.860
CB-187								1.000	0.834

and pups were released for approximately 4 h to allow equilibration of body water, then recaptured for a second blood sample to be taken (Reilly and Fedak 1990). Pups had been free to feed in this intervening period and were therefore reweighed.

All blood samples were centrifuged within 6 h of collection and stored at –20°C. Deuterated plasma was sealed in microcapilliaries on the day of collection for commercial mass spectrometry analysis (Centrum voor Isotopen Onderzoek, Groningen, the Netherlands). The water content of plasma samples was determined by drying weighed aliquots of plasma to constant mass. Relative concentrations of deuterated plasma samples were then used to calculate the size of pups' total body water (TBW) pool as described in Reilly and Fedak (1990).

Liquid scintillation spectrometry was used to determine the specific activity of tritiated plasma samples. Approximately 0.5 mg plasma was added to 10 mL of Instagel scintillation fluid (Packard Instruments, Caversham, U.K.) and counted for a minimum of 40 000 counts or 10 min in a refrigerated scintillation counter (Searle Mark 3, Uithon, the Netherlands). External standards were used to correct for quenching. Blanks of unenriched seal plasma were prepared and counted. Standards for the injectate were prepared by gravimetric dilution of unused injectate to levels similar to those of seals in vivo.

TBW pool size for mothers was calculated as described for grey seals in Reilly and Fedak (1990). Their relationship, %TBL = 105.1 – 1.47(TBW), where TBL is total body lipid, was used to calculate body lipid content of mothers and pups.

Our estimates of milk intake assume that milk is a pup's only external source of water. Therefore, any increases in pup body water are derived from preformed water in milk and from metabolic water production. Fractional water turnover can be estimated from the decline in corrected isotope concentration with time. Total water intake is the sum of gains and losses in the growing body, calculated from turnover and pool sizes. Milk intake can be calculated if the composition of milk over the course of the study is known.

Pups were weighed up to four times before weaning, but we avoided sampling females immediately after the birth of their pups to allow the mother–pup bond to establish normally. As a result we had to estimate birth and weaning masses. Pup mass at birth was calculated by assuming that pups did not gain mass in their 1st day and that growth was linear thereafter. Pups known or estimated to be more than 3 days old when they were first sampled were assigned the mean birth weight for pups at the Isle of May (males, 15.5 ± 0.40 kg (mean ± SE), $n = 31$; females, 15.2 ± 0.43 kg, $n = 31$). Pup mass at weaning was calculated using preweaning growth rate and postweaning loss rate extrapolated to the weaning date obtained from behavioural observations. Where isotope measurements were not available for part of the lactation period, intake of milk lipid over that period was estimated from the following relationship: milk lipid intake (kg) = 5.96 + 0.668(pup growth (kg)), $r^2 = 0.793$, $n = 42$.

Calculation of CB exposure

The quantity of CB transferred to a pup between two sampling dates

© 1996 NRC Canada

Fig. 4. Changes in the concentration of CB-118 in the milk of individual grey seals during the course of lactation. All concentrations are expressed in terms of the concentration of the CB in the first milk sample collected from each female in each year.

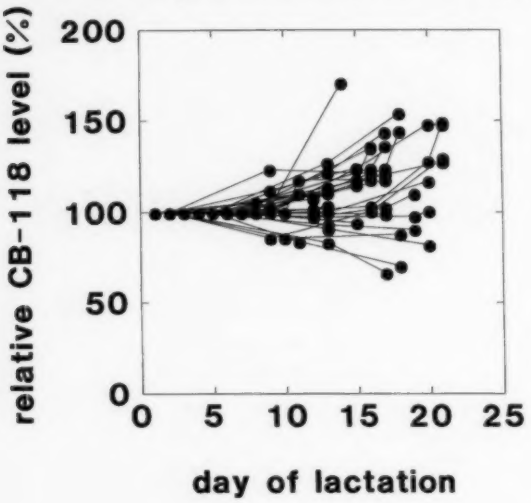
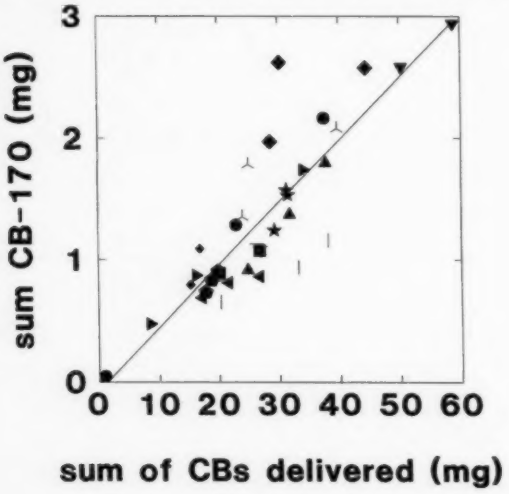


Fig. 5. Relationship between total exposure to CB-170 and exposure to the sum of all assayed CBs for grey seal pups. Values from the 13 individual mothers are identified by different symbols.



was calculated by multiplying the quantity of milk lipid received during that interval by the mean of the CB concentrations on the day of sampling and on the previous sampling. For the first and last samplings, the CB concentrations in milk collected on that day were used to calculate exposure during the early and last days of lactation. Daily exposure was calculated by dividing the amount of CB transferred by the interval between samples. Daily dose was defined as daily exposure divided by the mass of the pup at the midpoint of the interval. Total exposure to CB was calculated as the sum of the daily exposure values for the entire lactation period.

Fig. 6. Daily exposure to the sum of 25 individual CB congeners experienced by individual grey seal pups.

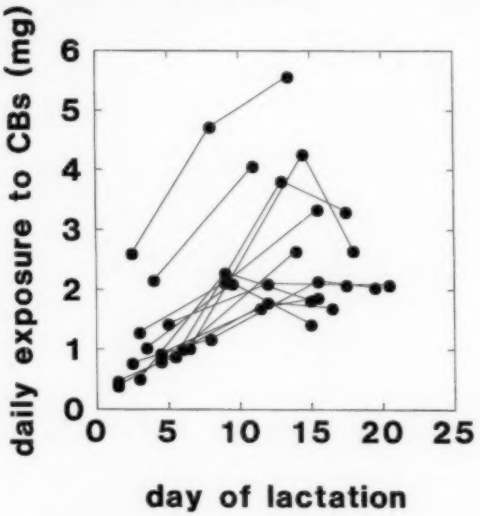
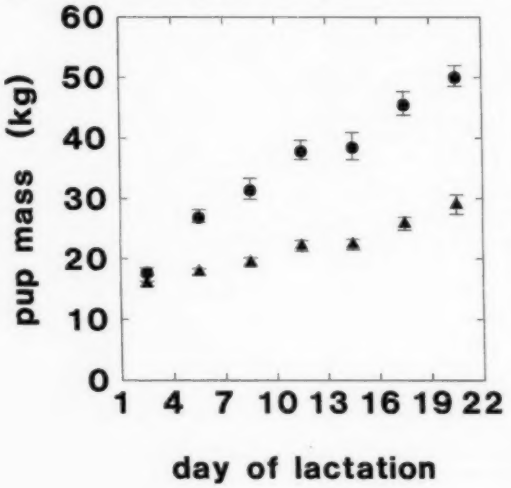


Fig. 7. Changes in the mean total body mass (●) and mean lean body mass (▲) of grey seal pups during the course of lactation.



Results

Study females were determined to be between 6 and 22 years old in 1990 (Table 1). Ten of the 13 females produced a pup in every year of the study. The mean duration of lactation was 17.3 ± 2.37 days ($n = 35$). One mother left her offspring prematurely in 2 of the 3 years. Using a generalized linear model (McCullagh and Nelder 1983) the mean weaning mass of pups produced in 1990 was lower than in 1991 or 1992 when account was taken of differences among females ($F_{[12,21]}$ mothers = 1.914, $P = 0.096$; $F_{[2,31]}$ year = 4.006, $P = 0.030$, $n = 35$).

© 1996 NRC Canada

Milk composition and intake by pups

The total amount of milk delivered by females to pups over the course of normal lactation varied between 27 and 69 kg (Table 1). Lipid content of milk increased from 38% of total milk mass at the beginning of lactation to a maximum of approximately 60% by day 12 (Fig. 1). Milk intake of pups varied among females, and within and among lactation periods for the same female. Mean milk intake increased from 2.39 ± 0.14 kg·day^{-1} (n = 18) on days 1–7 to 3.00 ± 0.22 kg·day^{-1} (n = 13) after day 15 (one-way analysis of variance, $F_{[1,29]}$ = 3.25, P = 0.05). As both lipid content and intake of milk increased during lactation, the amount of milk lipid delivered to pups also increased during the lactation period, reaching an asymptote at about day 8 (Fig. 2).

Concentration of CBs in milk

When concentrations are expressed as a proportion of total CB content, as suggested by Duinker et al. (1988), it is clear that the CB composition of all the milk samples was dominated by congeners CB-153, CB-138, CB-180, and CB-187 (Fig. 3). These CBs made up 23.9, 18.2, 14.1, and 8.8%, respectively, of the average milk total CB content. Concentrations of most congeners were highly correlated with each other and with the sum of all CBs (Table 2).

As there were variations in the concentrations of congeners in milks collected at different times from mothers, the relationship between the concentration of the 16 most abundant congeners and mother's identity, day of lactation, and year was investigated using a generalized linear model (Table 3). There were highly significant differences between mothers in the concentration of all congeners analysed. The less chlorinated congeners (CBs 52–138) also showed significant changes in concentration with day of lactation and year. However, there was no consistent pattern of variation in the concentrations of CBs in milk lipid during the course of a single lactation period for individual mothers. For example, the concentration of CB-118 standardized against the concentration of that congener in the first milk sample from each mother in each year increased during lactation for some mothers, whereas for others it decreased (Fig. 4). Therefore, it seems likely that the significant effects associated with year and day of lactation were the results of changes in the concentration of total CBs. When CB concentrations were expressed as a proportion of total CB content, day of lactation and year ceased to have a significant effect but the highly significant difference between mothers remained (Table 3).

Total exposure

The total quantities of CBs received by the pups of individual mothers varied between 8.8 and 58.9 mg (Table 1). There were temporal effects on CB exposures that were explained more by changing milk delivery than by changes in CB concentrations. The younger mothers in the study (0W, 1W, 7B, 9B, D6) delivered more lipid to pups in successive years whereas older mothers (D0, D1, H7, X+) did not show any trend. Of the exposures delivered by the 12 mothers in 1992, 6 were the highest recorded for these mothers, but 4 of these were the younger mothers delivering more milk, lipid, and therefore CBs than before.

Although total exposure to the sum of individual congeners was highly correlated with exposure to individual congeners, exposure to individual congeners varied significantly between mothers. For example, Fig. 5 shows the relationship between total exposure to CB-170 in milk and to the sum of all CBs in milk. The mean residuals around the regression line relating these points were different for individual mothers (one-way analysis of variance, $F_{[12,23]}$ = 8.845, P < 0.001). Most mothers produced exposures that were either consistently above or consistently below the regression line.

Daily exposure

Daily exposure to each congener increased during the lactation period, although for some pups exposure declined later in lactation (Fig. 6). The majority of pups born to study females fell within the same broad exposure band for each congener, but exposures from one female (2W) were up to an order of magnitude greater than these.

Daily dose

The dose of CBs received by each pup was determined by the daily exposure, itself dependent on mother and stage in lactation, and the pup's own body mass and composition. It is therefore important to chose an appropriate measure of body mass.

Although pups in this study increased their body mass by an average of 24.12 ± 2.33 kg during the course of lactation, most of that increase was in body lipid; the mean increase in lean body mass was only 9.55 ± 0.78 kg. The ratio of total body lipid to lean body mass increased linearly from a negligible amount at the start of lactation to values that approached unity near weaning. We believe that it is useful to express pup CB doses both per unit of total body mass and per unit of lean body mass to reflect the differences in pup body composition that occur (Fig. 7). Daily dose, expressed as mass of CBs delivered in milk per day per unit body mass, followed a similar trajectory through lactation whether expressed as total body mass or lean body mass (Fig. 8). However, daily dose expressed in terms of lean body mass increased more rapidly during the course of lactation. In the early part of lactation, pups were small and had little stored lipid, and the quantity of milk consumed and its lipid content was low. As a result, daily doses were relatively low. As milk consumption and milk lipid content increased during the course of lactation, daily dose also increased, reaching a maximum about the middle of the 18-day lactation period. Thereafter, the effects of increasing pup mass compensated for the increase in daily exposure. Towards the end of lactation, daily dose appeared to decline sharply. However, this is because the sample of animals with long lactation periods is biased towards large pups (see Table 1).

Discussion

There are few published accounts of the congener-specific composition of grey seal milk or other tissues. However, these accounts are in broad agreement with our results. Law et al. (1989) and Addison and Stobo (1993) reported that CB-153 and CB-138 were the dominant congeners in grey seal blubber samples, with CB-153 the most abundant congener. Boon and Eijgenraam (1988), Hall et al. (1992), and Storr-Hansen and Spliid (1993) obtained similar results for blubber and blood samples from North Sea harbour seals (*Phoca vitulina*). Schweigert and Knöppler (1990) also found that CB-153 and

© 1996 NRC Canada

Table 3. Generalized linear model analysis of CB congener concentration in milk lipid and relative to total CB concentration, with respect to mother, day of lactation, and year of sampling.

Variable		Mother	Day of lactation	Year	Multiple R^2
CB congener concentration in milk lipid					
CB-52	*F*	7.381	9.233	6.501	0.642
	p	<0.001	0.003	0.003	
CB-101	*F*	20.707	11.881	4.007	0.814
	p	<0.001	0.001	0.023	
CB-105	*F*	36.419	12.340	6.403	0.887
	p	<0.001	0.001	0.003	
CB-110/77	*F*	19.925	10.254	9.071	0.817
	p	<0.001	0.002	<0.001	
CB118	*F*	44.927	10.901	4.504	0.907
	p	<0.001	0.002	0.015	
CB-128	*F*	28.820	6.096	5.434	0.863
	p	<0.001	0.016	0.007	
CB-138	*F*	10.663	3.519	3.145	0.699
	p	<0.001	0.065	0.050	
CB-149	*F*	13.116	6.007	2.216	0.739
	p	<0.001	0.017	0.118	
CB-153	*F*	8.818	2.861	2.928	0.661
	p	<0.001	0.096	0.061	
CB-170	*F*	9.125	2.397	2.331	0.671
	p	<0.001	0.127	0.106	
CB-180	*F*	6.595	1.456	1.970	0.595
	p	<0.001	0.232	0.148	
CB-183	*F*	7.809	1.895	2.309	0.638
	p	<0.001	0.174	0.108	
CB-187	*F*	7.279	1.812	2.497	0.621
	p	<0.001	0.183	0.091	
CB-201	*F*	8.498	1.057	1.632	0.650
	p	<0.001	0.308	0.204	
CB-202	*F*	3.125	0.290	0.595	0.386
	p	<0.001	0.592	0.555	
Sum CBs	*F*	8.656	2.775	9.071	0.817
	p	<0.001	0.002	<0.001	
CB congener concentration relative to total CB concentration					
CB-52	*F*	5.482	0.011	0.228	0.517
	p	<0.001	0.918	0.796	
CB-101	*F*	19.54	0.487	0.874	0.793
	p	<0.001	0.488	0.423	
CB-105	*F*	17.76	0.825	0.106	0.777
	p	<0.001	0.367	0.900	
CB-110/77	*F*	10.213	1.854	2.107	0.677
	p	<0.001	0.178	0.130	
CB-118	*F*	2.869	0.184	0.240	0.378
	p	0.003	0.670	0.788	
CB-128	*F*	16.68	1.007	1.037	0.773
	p	<0.001	0.320	0.361	
CB-138	*F*	42.18	0.159	0.070	0.892
	p	<0.001	0.691	0.932	
CB-149	*F*	22.05	0.149	1.490	0.815
	p	<0.001	0.701	0.233	
CB-153	*F*	21.04	0.577	0.342	0.805
	p	<0.001	0.450	0.712	
CB-170	*F*	28.81	4.306	0.183	0.854
	p	<0.001	0.042	0.833	
CB-180	*F*	5.970	0.195	0.100	0.544
	p	<0.001	0.660	0.905	
CB-183	*F*	25.03	2.053	0.058	0.833
	p	<0.001	0.157	0.944	
CB-187	*F*	6.541	0.103	0.006	0.564
	p	<0.001	0.749	0.994	
CB-201	*F*	17.65	0.030	0.116	0.776
	p	<0.001	0.863	0.891	
CB-202	*F*	9.612	1.404	0.868	0.670
	p	<0.001	0.241	0.425	

CB-138 were the most abundant congeners in grey seal milk collected in Canada. However, in their samples, concentrations of CB-138 were higher than those of CB-153.

Our results show that grey seal pups born at the Isle of May received a cocktail of CB congeners in their mothers' milk; the composition of this cocktail varied significantly among females. This variation could have been due to a number of factors.

The contaminant burdens of adult female grey seals accumulate gradually in their lipid reserves until they give birth to and raise their first pup (Helle et al. 1976; Donkin et al. 1981); after this, burdens stabilize and may even decline (P.P. Pomeroy, N. Green, A.J. Hall, K. Jones, and J. Harwood, unpublished data). The size and composition of this burden will depend on the CB content of each seal's diet (cf. Reijnders 1986). Hammond et al. (1993) documented substantial variation in the foraging behaviour of individual grey seals in the North Sea; Prime and Hammond (1990) found significant regional and seasonal variations in the diet of grey seals in the same area. Individuals may also vary in their ability to metabolize certain congeners (Boon and Eijgenraam 1988).

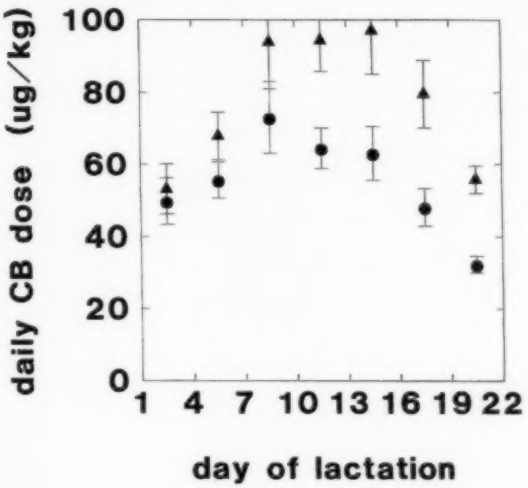
The CB composition of the milk produced by females will also depend on the way in which their lipid reserves are mobilized during lactation. Addison and Brodie (1977, 1987) found that the transfer of CBs from blubber to milk in grey seals on Sable Island, Nova Scotia, was selective. Highly chlorinated congeners, such as CB-180, occurred at lower levels in milk than in blubber. However, they found no selectivity in the movement of residues between blubber and blood.

The daily dose of CBs received by pups is affected by individual patterns of body mass change, as well as the quantity of CB delivered in their mothers' milk. If pups grow steadily, increases in body mass may match the increase in exposure to CBs that occurs during lactation so that the daily dose remains roughly constant. Pups that grow rapidly may experience high CB exposures but relatively low doses. Fast-growing pups tend to be born larger than average (Fedak and Anderson 1987; Bowen et al. 1992; Iverson et al. 1993), which will reduce the dose further. Conversely, small pups that grow slowly may receive higher than average doses, even if their mothers' milk contains only moderate concentrations of CBs. The effect of these different doses on the immune system of the study animals is discussed elsewhere (Hall et al. 1996).

Determinations of CB exposures and doses over a complete lactation period have allowed us to make comparisons between the pups born to the same females in different years. However, such comparisons assume that pups are fed entirely from the same female, usually their biological mother. However, we know that three of the pups in our study obtained milk from,

© 1996 NRC Canada

Fig. 8. Changes in mean daily dose of the sum of 25 individual CB congeners experienced by grey seal pups during the course of lactation. Dose is expressed in terms of total body mass (●) and lean body mass (▲).



or were fed by, more than one female. Two females (X+ and 9H) fed several pups in addition to their own in 1991. DNA analysis (P. Allen, Department of Genetics, University of Cambridge, Downing Street, Cambridge, U.K., personal communication) showed that in 1991 one female (7B) was feeding a pup that was not her own.

The methods we used were time consuming and required repeated capture and sampling of animals. We therefore addressed the following question: can results of acceptable accuracy (such as those required in immunocompetence studies) be obtained less laboriously? The best single predictor of total exposure to CBs was mother's identity, but even for this variable the correlation coefficient was relatively small (r^2 = 0.611). Other variables, such as CB concentration in the last milk sample (r^2 = 0.523) or pup mass at weaning (r^2 = 0.256), gave substantially lower values. A linear combination of mother's identity and milk CB content gave a value for r^2 of 0.761 with mother's identity becoming nonsignificant, and a combination of all three variables gave an r^2 value of 0.914, again with a nonsignificant contribution made by mother's identity. CB content in the last milk sample and pup mass at weaning were the best linear pair of exposure predictors (r^2 = 0.850); inclusion of duration of lactation improved the model slightly (r^2 = 0.877). Maternal variables such as mother's mass at parturition or mother's mass at weaning were not helpful in the regression equation. These results indicate that reliable estimates of the total quantities of CBs transferred by a particular female to her pup can be obtained from that pup's weight at weaning and information on the CB content of a milk sample collected towards the end of lactation. They demonstrate the value of long-term studies of individually marked animals.

The high correlations found between the concentrations of individual CB congeners in grey seal milk suggest that a congener-specific analysis is unnecessary. However, it would be misleading to draw this conclusion because individual females showed consistent deviations from the overall relationship between the concentrations of individual congeners. Values for total CB content derived from such relationships may be useful for broad comparisons between geographical areas. However, they are inappropriate if estimates of exposure are to be used in an ecotoxicological context. The toxic equivalence of individual congeners varies by several orders of magnitude (Ahlborg et al. 1994). Therefore, relatively small but consistent deviations from predictive regressions can result in biased estimates of the potential toxic effects of exposure, particularly for congeners like CB-170 (see Fig. 6), which had the highest toxic equivalence of all the congeners we measured (Ahlborg et al. 1994).

Our results demonstrate that the exposure of seal pups to individual CBs can be measured accurately in the field. They highlight that it is important to take account of the wide variation between individual mothers in the quantities and composition of CBs they deliver to their pups.

Acknowledgments

The authors thank those who assisted in fieldwork, particularly S. Twiss and S. Licence. We thank R.F. Addison and an anonymous referee for comments that improved the paper. This research was made possible by a contract from Rechem Environmental Services PLC to the Natural Environment Research Council Sea Mammal Research Unit. Aspects of the work were funded under contract to the U.K. Department of the Environment as a contribution to its coordinated programme of research on the North Sea.

References

Addison, R.F., and Brodie, P.F. 1977. Organochlorine residues in maternal blubber, milk and pup blubber from grey seals, *Halichoerus grypus*, from Sable Island, Nova Scotia. J. Fish. Res. Board. Can. **34**: 937–941.

Addison, R.F., and Brodie, P.F. 1987. Transfer of organochlorine residues from blubber through the circulatory system to milk in the lactating grey seal *Halichoerus grypus*. Can. J. Fish. Aquat. Sci. **44**: 782–786.

Addison, R.F., and Stobo, W.T. 1993. Organochlorine residue concentrations and burdens in grey seal (*Halichoerus grypus*) blubber during the first year of life. J. Zool. (London), **230**: 443–450.

Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Glor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Schlatter, C., Waern, F., Younes, M., and Yrjanheikki, E. 1994. Toxic equivalency factors for dioxin-like PCBs: report on a WHO-ECEH and IPCS consultation, December 1993. Chemosphere, **28**: 1049–1067.

Bacon, C.E., Jarman, W.M., and Costa, D.P. 1992. Organochlorine and polychlorinated biphenyl levels in pinniped milk from the Arctic, the Antarctic, California and Australia. Chemosphere, **24**: 779–791.

Baker, J.R. 1990. Grey seal (*Halichoerus grypus*) milk composition and its variation over lactation. Br. Vet. J. **146**: 233–238.

Baker, J.R., Fedak, M.A., Anderson, S.S., Arnbom, T., and Baker, R. 1990. Use of a tiletamine–zolazepam mixture to immobilise wild grey seals and southern elephant seals. Vet. Rec. **126**: 75–77.

Ballschmitter, K., and Zell, M. 1980. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Fresenius Z. Anal. Chem. **302**: 20–31.

Boon, J.P., and Eijgenraam, F. 1988. The possible role of metabolism

© 1996 NRC Canada

in determining patterns of PCB congeners in species from the Dutch Wadden sea. Mar. Environ. Res. **24**: 3–8.

Bowen, W.D., Stobo, W.T., and Smith, S.J. 1992. Mass changes of grey seal *Halichoerus grypus* pups on Sable Island: differential maternal investment reconsidered. J. Zool. (London), **227**: 607–622.

Carter, S.D., Hughes, D.E., and Baker, J.R. 1990. Characterization and measurement of immunoglobulins in the grey seal (*Halichoerus grypus*). J. Comp. Pathol. **102**: 13–23.

de Swart, R., Ross, P.S., Vedder, L.J., Timmerman, H.H., Heisterkamp, S., van Loveren, H., Vos, J.G., Reijnders, P.J.H., and Osterhaus, A.D.M.E. 1994. Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. Ambio, **23**: 155–159.

Donkin, P., Mann, S.V., and Hamilton, E.I. 1981. Polychlorinated biphenyl, DDT and dieldrin residues in grey seal (*Halichoerus grypus*) males, females and mother–foetus pairs sampled at the Farne Islands, England, during the breeding season. Sci. Total Environ. **19**: 121–142.

Duinker, J.C., and Hillebrand, M.T.J. 1983. Composition of PCB mixtures in biotic and abiotic marine compartments (Dutch Wadden Sea). Bull. Environ. Contam. Toxicol. **31**: 25–32.

Duinker, J.C., Knap, A.H., Brinkley, K.C., Van Dam, G.H., Darrel-Rew, A. and Hillebrand, M.T.J. 1988. Method to represent the qualitative and quantitative characteristics of PCB mixtures: marine mammal tissues and commercial mixtures as examples. Mar. Pollut. Bull. **19**: 2, 74–79.

Fedak, M.A., and Anderson, S.S. 1987. Estimating the energy requirements of seals from weight changes. *In* Approaches to marine mammal energetics. Society for Marine Mammology special publication No. 1. *Edited by* A.C. Huntley, D.P. Costa, G.A.J. Worthy, and M.A. Castellini. Allen Press, Lawrence, Kans. pp. 205–226.

Hall, A.J., Law, R.J., Wells, D.E., Harwood, J., Ross, H., Kennedy, S., Allchin, C.R., Campbell, L.A., and Pomeroy, P.P. 1992. Organochlorine levels in common seals (*Phoca vitulina*) which were victims and survivors of the 1988 phocine distemper epizootic. Sci. Total Environ. **115**: 145–162.

Hall, A.J., Pomeroy, P.P., Green, N., Jones, K., and Harwood, J. 1996. Infection, haematology, and biochemistry in gray seal pups exposed to chlorinated biphenyls. Mar. Envir. Res. **44**: 1–18.

Hammond, P.S., McConnell, B.J., and Fedak, M.A. 1993. Grey seals off the east coast of Britain: distribution and movements at sea. Symp. Zool. Soc. Lond. **66**: 211–224.

Helle, E., Olsson, M., and Jensen, S. 1976. PCB levels correlated with pathological changes in seal uteri. Ambio, **5**: 261–263.

Iverson, S.J., Bowen, W.D., Boness, D.J., and Oftedal, O.T. 1993. The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). Physiol. Zool. **66**: 61–88.

Kovacs, K.M., and Lavigne, D.M. 1986. Maternal investment and neonatal growth in phocid seals. J. Anim. Ecol. **55**: 1035–1051.

Law, R.J., Allchin, C.R., and Harwood, J. 1989. Concentrations of organochlorine compounds in the blubber of seals from eastern and north-eastern England, 1988. Mar. Pollut. Bull. **20**: 110–115.

McCullagh, P., and Nelder, J.A. 1983. Generalized linear models. Monogr. Stat. Appl. Probab.

Oftedal, O.T., Iverson, S.J., and Boness, D.J. 1987. Milk and energy intakes of suckling California sea lion, *Zalophus californianus*, pups in relation to sex, growth and predicted maintenance requirements. Physiol. Zool. **60**: 560–575.

Prime, J.H., and Hammond, P.S. 1990. The diet of grey seals from the south-western North Sea assessed from analyses of hard parts found in faeces. J. Appl. Ecol. **27**: 435–447.

Reijnders, P.J.H. 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature (London), **324**: 456–457.

Reilly, J.J., and Fedak, M.A. 1990. Measurement of the body composition of living gray seals by hydrogen isotope dilution. J. Appl. Physiol. **69**: 885–891.

Ross, P.S., Pohajdak, B., Bowen, W.D., and Addison, R.F. 1993. Immune function in free-ranging harbour seal (*Phoca vitulina*) mothers and their pups during lactation. J. Wildl. Dis. **29**: 21–29.

Schweigert, V.F.J., and Knöppler, H.-O. 1990. Polychlorierte biphenyle im Depotfett von Kegelrobben (*Halichoerus grypus*) und deren Transfer vom Muttertier mit der Milch zum Säugling. Arch. Lebensmittelhyg. **41**: 79–89.

Storr-Hansen, E., and Spliid, H. 1993. Coplanar polychlorinated biphenyl congener levels and patterns and the identification of separation populations of harbor seals (*Phoca vitulina*) in Denmark. Arch. Environ. Contam. Toxicol. **24**: 44–58.

© 1996 NRC Canada

Methyl mercury production in freshwater microcosms affected by dissolved oxygen levels: role of cobalamin and microbial community composition

Olof Regnell, Anders Tunlid, Göran Ewald, and Olof Sangfors

Abstract: Radiolabelled $Hg(NO_3)_2$ was added to microcosms containing water and sediment from a lake contaminated with cellulose fibres and mercury. We found that anoxic incubation resulted in methyl ^{203}Hg concentrations in the water at least an order of magnitude higher than the levels in oxygenated incubation. This was explained by the fact that both the production and the water solubility of methyl mercury were higher under anoxic conditions than under oxic conditions. Demethylation, however, seemed to play a minor role, if any, in regulating methyl mercury concentrations. In the sediment, the level of methyl ^{203}Hg was strongly correlated with the level of cobalamin and the levels of the phospholipid fatty acids i15:0 and 10Me16:0, the latter indicating the presence of *Desulfobacter*, a genus of sulphate-reducing bacteria.

Résumé : Du $Hg(NO_3)_2$ radiomarqué a été introduit dans des microcosmes contenant de l'eau et des sédiments provenant d'un lac contaminé par des fibres de cellulose et du mercure. Nous avons trouvé que l'incubation anoxique produisait des concentrations dans l'eau de méthyl ^{203}Hg plus élevées d'au moins un ordre de grandeur que dans le cas de l'incubation sous oxygène. Cela s'explique par le fait que tant la production que la solubilité dans l'eau du méthylmercure étaient plus élevées dans des conditions anoxiques que dans des conditions oxiques. La déméthylation ne semblait toutefois jouer qu'un rôle mineur ou nul dans la régulation des concentrations de méthylmercure. Dans le sédiment, la concentration de méthyl ^{203}Hg était fortement corrélée à la teneur en cobalamine et aux concentrations d'acides gras des phospholipides i15:0 et 10Me16:0, ce dernier élément indiquant la présence de *Desulfobacter*, genre de bactéries réductrices des sulfates.
[Traduit par la Rédaction]

Introduction

High levels of methyl mercury in fish pose a threat to fish consumers. Mercury pollution is a rather common problem, unfortunately, although various countries have prohibited or restricted the use of mercury in industrial processes and in agriculture.

Most methyl mercury production in aquatic environments is probably a result of microbial activity (Craig and Moreton 1985). Since the demonstration by Compeau and Bartha (1985) that sulphate-reducing bacteria are the main methylators of mercury in anoxic estuarine sediments, evidence has been provided that this might be the case in other anoxic aquatic environments as well (Gilmour and Henry 1991; Gilmour et al. 1992). It has long been suspected that methyl cobalamin (a vitamin B_{12} derivative) is involved in the microbial methylation of mercury, since this compound is known to efficiently donate methyl groups to inorganic Hg(II) (Bertilsson and Neujahr 1971; Craig and Moreton 1985). Recently, it was shown that methyl cobalamin is indeed the methyl group donor when inorganic Hg(II) is methylated by *Desulfovibrio desulfuricans*, a sulphate reducer (Choi and Bartha 1993). However, there have been no reports of a positive relationship between cobalamin levels and mercury methylation in environmental samples, nor does such a relationship seem very likely considering the fact that a large number of different microorganisms contain high levels of cobalamin, including those that so far have not been associated with mercury methylation, e.g., homoacetogens and methanogens (Choi et al. 1994). Decomposition of methyl mercury (demethylation) is also mediated by microorganisms (Oremland et al. 1991; Robinson and Tuovinen 1984; Spangler et al. 1973). The fact that methyl mercury disappears from lake water when anoxic bottom water is reoxygenated during turnover events (Bloom and Effler 1990) may be an indication that demethylation occurs primarily under oxygenated conditions. Indeed, demethylation has been shown to proceed at a faster rate in oxygenated sediment than in anoxic sediment (Compeau and Bartha 1984; Ramlal et al. 1986). However, other mechanisms of removal of methyl mercury from the water cannot be excluded, one being redox-controlled uptake of methyl mercury by the sediment (Regnell 1995).

In our laboratory we have developed aquatic microcosms containing undisturbed sediment and water that allow us to study simultaneously the methylation and volatilization of mercury under natural conditions (Regnell 1994; Regnell and Tunlid 1991). Besides the analysis of mercury speciation and partitioning, various ancillary variables can also be measured,

Received May 25, 1995. Accepted January 19, 1996.
J12930

O. Regnell,[1] A. Tunlid, and G. Ewald. University of Lund, Department of Ecology, Ecology Building, S-223 62 Lund, Sweden.
O. Sangfors. The Swedish Environmental Research Group, Fryksta, S–665 00 Kil, Sweden.

[1] Author to whom all correspondence should be addressed. e-mail: Olof.Regnell@ecotox.lu.se

© 1996 NRC Canada

Table 1. Some basic characteristics of the sediment and water used in the experiment.

	Fibre deposit	Outside fibre deposit
Sediment, 0–1 cm		
Total Hg (ng/g dry weight)	1085±142	1317±69
Organic matter content (% loss on ignition)	26±0.3	31±0.4
Water		
Depth (m)	4	14
In situ temperature (°C)	2.2	3.4
pH	6.08	6.05
Alkalinity (μequiv./L)	78	107

including those associated with the composition of the microbial community and the cobalamin content of the sediment and water. In the present study, phospholipid fatty acid (PLFA) analysis was used to characterize the microbial community. More specifically, the total amount of PLFA was used as a measure of the microbial biomass, and individual signature PLFAs were used to identify specific groups of bacteria (White 1988). Since this method of analysing the microbial composition is not dependent on growth, it does not have the problem of microbial selection inherent in the enrichment techniques.

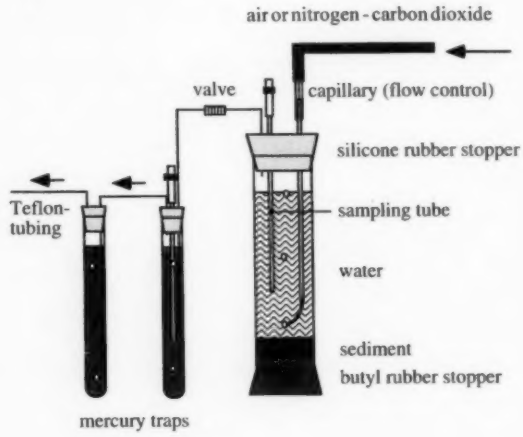
In a previous study employing the techniques described above, we found no correlation between cobalamin content and mercury methylation, nor between the composition of PLFA and mercury methylation (Regnell and Tunlid 1991). However, we felt that there was a need to examine whether such relationships might develop if the incubation time was increased and more adequate sampling of the water and sediment was performed. Furthermore, a turnover event was simulated in the microcosms by changing the oxygen regime, thus making it possible to determine whether methyl mercury that accumulated in the water under anoxic conditions disappeared under well-oxygenated conditions owing to increased demethylation, generating volatile elemental mercury. The change in oxygen regime ensured that all sediment cores were subject to both anoxic and oxygenated conditions and thus were comparable at the end of incubation. We decided to use sediment and water from a lake polluted with cellulose fibres and mercury, since the fate of mercury in such sites is of great concern in Sweden and probably elsewhere.

Materials and methods

Field sampling

In April 1991, sediment cores with overlying water were collected in acrylic plastic cylinders (inside diameter 69 mm; length 600 mm) from a forest lake in central Sweden (Lake Grycken), which receives effluent from a pulp mill. Until the mid 1960s the effluent contained fibres contaminated with phenyl mercuric acetate. A fibre deposit still covers 20 ha or 8% of the total lake area. Using a gravity corer of our own design, we took eight sediment cores at a depth of 4 m within this area and two sediment cores outside it at a depth of 14 m. The cylinders were closed with butyl rubber stoppers at the bottom and silicone stoppers at the top. The sediment cores were reduced to 5 cm, as described previously (Regnell and Tunlid 1991), with minimal disturbance of the sediment layers. The overlying water columns were then adjusted to 45.5 cm with water from the sampling site, the water making up a volume of 1700 mL. The cores were stored in a refrigerated box during transport to the laboratory, where they were placed in a water bath at 13 ± 0.5°C and kept in darkness. The temperature was increased relative to the in situ temperature to obtain a higher level of microbial activity. Table 1 indicates various sediment and water characteristics at the time and site of sampling.

Fig. 1. The design of the microcosms used to study chemical speciation and partitioning of mercury in lake sediment and water. The mercury traps contained 0.1 M $K_2Cr_2O_7$ dissolved in 1 M HNO_3.



Experimental design

After being stored in the laboratory for a day, the cylinders containing the cores were connected to a gas flow system that aerated the water columns of five of the cylinders (one of which contained a sediment core without cellulose fibres) and supplied N_2 and CO_2 to the other five (the partial pressure of CO_2 being 0.03%) (Fig. 1). The flow rate was kept constant by means of capillaries and a pressure regulator.

After a stabilization period of 1 week, the cylinders were disconnected from the gas supply and 784 kBq $^{203}Hg(NO_3)_2$ dissolved in 25 μL of 0.5 M HNO_3 was added to the water surface in each of the cylinders. Since the specific activity was 50 kBq/μg, the mercury addition corresponded to 9 μg of Hg/L. Five hours after the addition of mercury, the cylinders were reconnected to the gas supply. The outflow passed through mercury traps containing 0.1 M $K_2Cr_2O_7$ in 1 M HNO_3 (Fig. 1).

On day 37, the oxygen regimes were reversed so that the anoxic microcosms became oxygenated, and vice versa. The new oxygen regime prevailed until the microcosms were removed for final sampling, anoxic and oxygenated microcosms alternately, between days 65 and 88. For practical reasons, day 78 (the average total incubation time) is designated as the day of final sampling in the figures and tables.

Sampling of microcosms

Water from the microcosms as well as the dichromate solutions from the traps was withdrawn through the sampling tubes by a plastic syringe (Fig. 1). Filtered water samples were obtained by passing the water through 0.2-μm filters. When the upper stoppers were opened at the end of the two oxygen regimes, large water samples (200 mL) were taken for phospholipid fatty acid analyses. At the end of the first oxygen regime (day 37), lake water that was sampled at the same sites as the sediment cores and then stored at 5°C was added to all of the microcosms to replace the water that was withdrawn. The resulting dilution was compensated for when ^{203}Hg concentrations in the water, before and after the addition of new water, were compared.

After the final sampling of the water, all of the water was removed

© 1996 NRC Canada

and the upper 0–1 cm of the sediment layer and the sediment at 1–5 cm depth were transferred to separate centrifuge tubes. The sediment was centrifuged at 8000 × *g* to remove water from the sediment.

Extractable ^{203}Hg and methyl ^{203}Hg determinations

Extractable Hg was defined operationally as the fraction of the total Hg that was partitioned into benzene or toluene from water and sediment samples that had previously been shaken with $CuSO_4$ and acidic bromide solutions and been left to stand for 30 min (Regnell and Tunlid 1991). To determine the methyl ^{203}Hg fraction of the extractable ^{203}Hg, extracts were applied to thin-layer chromatography plates (silica gel 60, F254; Merck) together with a methyl mercury bromide standard. The plates were eluted with either hexane–acetone (3:2) or chloroform–methanol (9:1). The same results were obtained with both elution mixtures, although the R_f values for methyl mercury bromide differed, being 0.5 for hexane–acetone (3:2) and 0.7 for chloroform–methanol (9:1). Methyl mercury spots were made visible by spraying the plates with 0.05% diphenylthiocarbazone in chloroform. The spots identified in this way were removed with a spatula, and their ^{203}Hg activity was determined.

Measurements of ^{203}Hg activity

The ^{203}Hg activity was determined using a gamma counter (LKB Wallac 1282 Compugamma). Sediment (0.5–1 g) and water (1 mL) were placed in plastic scintillation vials (5 mL), and 3 mL of 1 M HCl was added to the water samples. The sediment samples were suspended in distilled water, each making up a total volume of 4 mL. Sediment and water extracts in toluene or benzene (3.5 mL) were placed in glass scintillation vials (4 mL). The counting efficiencies for plastic and glass vials were 28 and 32%, respectively. The measured activities were corrected accordingly, as well as for radioactive decay.

Dissolved oxygen, sulphide, and pH

An oxygen electrode (Orion 97–08) equipped with a measuring cell of our own design was used for all dissolved oxygen measurements. Water samples were withdrawn from the microcosms using a glass syringe and immediately transferred to the measuring cell. Dissolved oxygen levels in oxygenated microcosms were at 100% saturation but were below the detection limit (0.1 mg/L) in anoxic microcosms. Only the day after the change in oxygen regime did the water in the microcosms show intermediate oxygen levels.

Sulphide in the water was determined by direct iodometry. Ten-millilitre water samples were withdrawn and then transferred to screw-cap test tubes containing 1 mL of 0.005 M iodine solution. The samples were allowed to stand for 15 min and were then titrated with 0.002 M $Na_2S_2O_3$. The difference in the consumption of $Na_2S_2O_3$ between blanks (10 L of distilled water added to 1 mL of 0.005 M iodine solution) and the samples was used to calculate the sulphide concentration. Sulphide levels in the water of anoxic microcosms, measured before and after the change in oxygen regime, were 1.5 ± 0.5 mg/L (on day 33) and 1.4 ± 0.7 mg/L (on day 61). No sulphide was detected in the water of oxygenated microcosms.

The pH of the water was measured with a pH meter (Hanna Instruments 8424). Initially, the pH of the water was 6.5–7.1, but during the incubation it increased to 6.8–7.6. The average pH values of the water in oxygenated and anoxic microcosms were not significantly different.

Cobalamin

Approximately 100 mg of lyophilized sediment was transferred to screw-cap test tubes, and 10 mL of 0.1 M acetate buffer (pH 4.6) containing 3 µM KCN was added (Osterdahl et al. 1985). The test tubes were placed in a boiling water bath for 30 min and were then centrifuged at 1500 × *g*, whereupon 200 µL of the supernatant was withdrawn for analysis.

Unfiltered water samples (100 mL) were acidified to pH 4 with HCl, and KCN was added to a final concentration of 3 µM. The acidified samples were preconcentrated on SPE columns (C18, 100 mg, Bond Elut LRC, Varian). Before the samples were applied, the columns were preconditioned with 2 × 2 mL of methanol followed by 2 × 2 mL of deionized distilled water. The columns were washed with 2 ×1 mL deionized distilled water after which cobalamin was eluted with 2 × 0.5 mL of *t*-butanol – water (1:5); 200 µL of the *t*-butanol extract was used in the analysis. It was confirmed by standard additions that no breakthrough of cobalamin occurred and that elution was complete.

A vitamin B_{12} – folate dual radioimmunoassay (RIA) kit (Radiochemical Centre, Amersham England) was used to determine the concentration of cobalamin, following the manufacturer's instructions.

Bacteria

Bacteria in the water (2-mL samples) were counted as described by Porter and Feig (1980). Formalin (final concentration 2%) was used for fixation and 4,6-diamidino-2-phenylindole (10 µg/mL) for staining the bacterial cells. The cells were collected on precolored 0.2-µm pore size polycarbonate membrane filters (Poretics, Livermore, Calif.) and were counted using an epifluorescence microscope.

Phospholipid fatty acid analysis

Lyophilized sediment was extracted with a chloroform–methanol–water mixture (Bligh and Dyer 1959). The water samples (200 mL) were filtered through preextracted polyvinylidene difluoride 0.2-µm filters (Millipore). These were then extracted overnight with *n*-hexane – isopropanol – water (Guckert and White 1988). The lipid extracts were fractionated by means of silicic acid chromatography (Tunlid et al. 1989). The polar lipid fractions, which contained phospholipids, were then transesterified (White et al. 1979), and the methyl esters of fatty acids were analysed by capillary gas chromatography (GC) using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a 50-m HP5 capillary column (phenyl methyl silicone; Hewlett-Packard Co., Palo Alto, Calif.). The initial oven temperature was 80°C. After 1 min, it was increased by 20°C/min to 160°C and then by 5°C/min to 270°C, at which it was held for 5 min. The injector and the detector temperatures were 230 and 270°C, respectively. Hydrogen was used as the carrier gas. Peak areas were quantified by adding methyl nonadecanoate as an internal standard before the transesterification step.

Mass spectrometric analysis was performed with a Hewlett-Packard 5970 GC – mass spectrometry system. The GC conditions were those described above, but helium was used as the carrier gas. The electron energy in the electron impact was 70 eV. Identification of fatty acid methyl esters was based on comparisons with spectra obtained from standards or from the literature (Ryhage and Stenhagen 1960). The position of double bonds was determined by analysing the dimethyldisulphide adducts (Nichols et al. 1986).

The PLFAs are designated in terms of total number of carbon atoms followed by the number of double bonds, the position closest to the aliphatic end of the molecule being indicated. The geometry of the double bond is indicated by c for cis and t for trans. The prefix Br indicates a methyl branch of unknown position. Unsaturated fatty acids having double bonds of unknown position are distinguished by the suffixes a, b, c, and so forth.

Data analysis

Results from measurements in the anoxic and oxygenated microcosms are given as the mean ± 1 standard deviation. Student's *t* test was used for comparisons of means of independent observations. Before significance probabilities were computed (*P* values), variables expressing concentrations were log-transformed and variables expressing fractions were arcsine-transformed (Ott 1990; Sokal and Rohlf 1981). To study relationships between variables, correlation coefficients (*r*) and *P* values were computed for simple linear log-log regression lines. $P < 0.05$ was chosen as the limit of statistical significance.

© 1996 NRC Canada

Table 2. The total ^{203}Hg concentrations and the particulate proportions of total ^{203}Hg and extractable ^{203}Hg in the water of the microcosms at different stages during incubation.

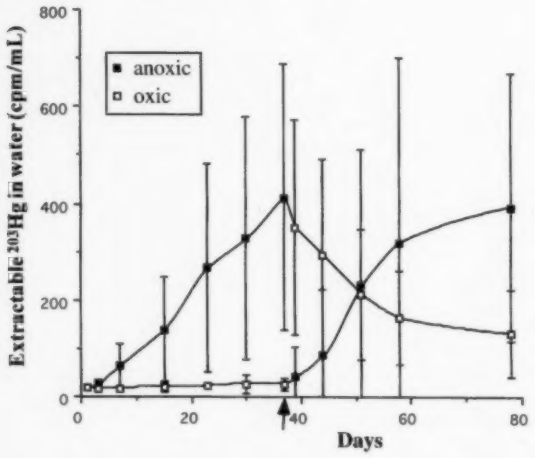
	Aerobic[a]	Anaerobic[a]	P^b
Day 1			
Total ^{203}Hg (cpm/mL)	22 582 ± 897	22 110 ± 1217	0.5
Day 37			
Total ^{203}Hg(cpm/mL)	6 124 ± 1222	10 791 ± 2116	0.003
Particulate total ^{203}Hg (%)	40 ± 11	39 ± 5	0.9
Particulate extractable ^{203}Hg (%)	46 ± 17	16 ± 16	0.05
Day 78			
Total ^{203}Hg (cpm/mL)	4 957 ± 1041	4 254 ± 992	0.2
Particulate total ^{203}Hg (%)	31 ± 16	38 ± 18	0.5
Particulate extractable ^{203}Hg (%)	32 ± 10	10 ± 9	0.006

Note: The particulate proportions are expressed as percentages of total ^{203}Hg and extractable ^{203}Hg, respectively, in unfiltered water. Values are given as averages ± 1 standard deviation (n = 5). On day 37, a reversal of the oxygen regimes was imposed so that anaerobic microcosms became aerobic and vice versa. Thus, the anaerobic micrososms on day 37 were the aerobic microcosms on day 78, and the aerobic microcosms on day 37 were the anaerobic microcosms on day 78.

[a]Refers to the water column.

[b]Comparison of means using Student's t test.

Fig. 2. The concentration of extractable ^{203}Hg in the water under aerobic and anaerobic conditions versus incubation time. The arrow indicates when the oxygen regime was changed. Bars indicate ±1 standard deviation (n = 5).



Principal component analysis (PCA) was performed on the PLFA composition in the sediment to elucidate differences in the composition of microorganisms between fibre sediment and fibreless sediment, and between incubated sediment and in situ sediment. The relative concentrations of the individual PLFAs, expressed as mole percent, were log-transformed prior to multivariate calculations. The computer program SIRIUS (Kvalheim and Karstang 1987) was used for all of the multivariate calculations and the corresponding graphical presentations.

Results

Speciation and partitioning of ^{203}Hg

At the end of the experiment the added ^{203}Hg was distributed as follows: 8 ± 2% (n = 10) of it was found in the water, 56 ± 12% (n = 10) in the 0- to 1-cm sediment layer, 9 ± 4% (n = 10) in the 1- to 5-cm sediment layer, and 0.4 ± 0.2% (n = 10) in the mercury traps (volatilized ^{203}Hg). Taking into account that water samples withdrawn during the experiment corresponded to an additional 5% of the added ^{203}Hg, we calculated the total recovery of the ^{203}Hg added to the microcosms to be 79 ± 12% (n = 10). We assume that the ^{203}Hg unaccounted for was adsorbed by the cylinder walls, since no leakage occurred during the experiment.

Before the change in oxygen regime, the concentration of extractable ^{203}Hg had become significantly higher in the anoxic water than in the oxygenated water (Fig. 2, Table 2). Methyl ^{203}Hg was found to constitute 80–90% of the extractable ^{203}Hg in unfiltered anoxic water but only 40–60% of the extractable ^{203}Hg in unfiltered oxygenated water. At the end of the first oxygen regime (day 37), 3.1 ± 1.8% (n = 5) of the total ^{203}Hg was methyl ^{203}Hg in the water of the anoxic microcosms and 0.3 ± 0.2% (n = 5) was methyl ^{203}Hg in the water of the oxygenated microcosms. The absolute difference in methyl ^{203}Hg concentration between the anoxic and the oxygenated microcosms was larger than suggested from the percentages above, since the retention time of total ^{203}Hg was significantly prolonged in the anoxic microcosms relative to the oxygenated microcosms (Table 2).

The proportion of total ^{203}Hg that was in the particulate fraction did not differ significantly between the anoxic and oxygenated microcosms. However, a significantly higher proportion of extractable ^{203}Hg was particulate (collected by 0.2-μm filters) in microcosms that were kept oxygenated than in microcosms that were kept anoxic (Table 2).

After the change in oxygen regime, the concentration of extractable ^{203}Hg declined in the water of the microcosms that became oxygenated and increased in the water of the microcosms that became anoxic (Fig. 2). During the second oxygen regime, volatilization of ^{203}Hg could only explain 19 ± 11% (n = 5) of the loss of methyl ^{203}Hg from the water of the oxygenated microcosms. In these microcosms 2.5 ± 1.9% (n = 5) of the total ^{203}Hg in the water was methyl ^{203}Hg at the end of incubation compared with 9.0 ± 9.3% (n = 5) in the anoxic

© 1996 NRC Canada

Fig. 3. The sum of the concentrations of all identified phospholipid fatty acids (PLFAs) in the water versus the density of bacteria in the water at the end of the second oxygen regime. Each symbol represents one microcosm. (One sample was analysed in each microcosm.)

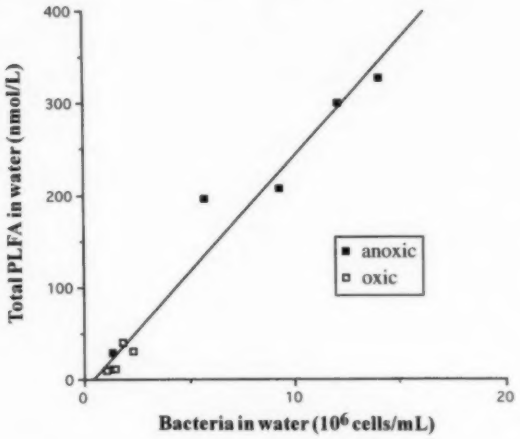


Table 3. Cobalamin concentrations in the sediment and in the water of the microcosms.

Sample	Cobalamin concentration		
	Aerobic[a]	Anaerobic[a]	P^b
Water			
Day 23	25±14	9±3	0.04
Day 37	20±13	5±2	0.02
Day 78[c]	25±16[d]	16±9	0.3
Sediment			
0–1 cm[c]	520±196	493±208	0.8
1–5 cm[c]	408±225	413±215	0.9

Note: Values are given as the average ± 1 standard deviation ($n = 5$). Cobalamin concentrations are given in picomoles per litre for water and in picomoles per gram dry weight for sediment. On day 37, a reversal of the oxygen regimes was imposed so that anaerobic microcosms became aerobic and vice versa. Thus, the anaerobic micrososms on day 37 were the aerobic microcosms on day 78, and the aerobic microcosms on day 37 were the anaerobic microcosms on day 78.

[a]Refers to the water column.

[b]Comparison of means using Student's t test.

[c]At the end of the second oxygen regime.

[d]Significantly higher than on day 37 ($P < 0.001$, paired t test).

microcosms. (These calculations were made assuming that the ratio between methyl ^{203}Hg and extractable ^{203}Hg was the same in the water as in the sediment at the end of incubation.)

In the 0- to1-cm sediment layers, 2.3 ± 2.2% ($n = 10$) of the total ^{203}Hg was methyl ^{203}Hg. The oxygen regime (whether the microcosms were oxygenated at the start or at the end of incubation) did not significantly affect the methyl and extractable ^{203}Hg concentration in the sediment ($P > 0.7$, and $P > 0.9$, t test). At the end of the experiment, the amount of extractable ^{203}Hg in the 0- to 1-cm sediment layers was on average 3 times larger than in the water (data not shown).

The volatilization of ^{203}Hg did not differ significantly between oxygenated and anoxic microcosms. However, evaluation of this was problematic, since ^{203}Hg was apparently lost from traps connected to anoxic microcosms (data not shown). Later, in separate experiments, it was found that H_2S precipitated ^{203}Hg in the trap solutions, and that this precipitate was adsorbed by the wall of the trap. This probably explained the apparent loss of ^{203}Hg from the traps connected to anoxic systems and the finding that ^{203}Hg still was not found in the traps second in line. Furthermore, it was found that the precipitate redissolved when the traps were bubbled with air instead of H_2S. Thus, the increase in the concentrations of ^{203}Hg in the traps following the change from anoxic to oxygenated conditions was probably not due entirely to newly formed Hg^0 but were also due to dissolution of precipitated ^{203}Hg already present in the traps.

Bacterial biomass

There was a strong correlation between bacterial density and the sum of the concentrations of the different PLFAs in the water (Fig. 3). Note that both of these estimates indicated higher biomass for anoxic water than for oxygenated water (Fig. 3). However, in the sediment the sum of the concentrations of the different PLFAs was not significantly different in microcosms subjected to reverse oxygen regimes ($P > 0.9$, t test).

Cobalamin

Before the change in oxygen regime the concentrations of cobalamin in the water (measured on days 23 and 37) were higher in the oxygenated microcosms than in the anoxic ones. Furthermore, there was a significant increase in cobalamin concentration when the anoxic microcosms were oxygenated (Table 3). However, the oxygen regime (whether the microcosms were oxygenated at the start or at the end of incubation) did not significantly affect the cobalamin concentration in the sediment (Table 3).

Extractable ^{203}Hg and methyl ^{203}Hg versus bacterial community structure and cobalamin concentrations

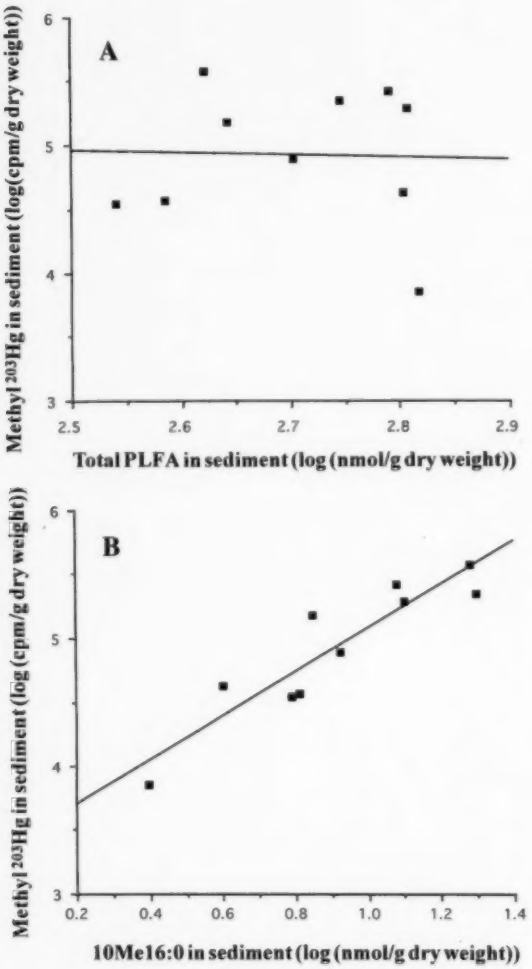
The sum of the concentrations of the different PLFAs in anoxic water, measured at the end of the first oxygen regime (day 37) and at the end of second oxygen regime (day 78), was not significantly correlated with the concentration of extractable ^{203}Hg (Table 4). However, individual PLFAs showed significant positive correlation with extractable ^{203}Hg in anoxic water. Of these, 18:1ω7t was significantly correlated with extractable ^{203}Hg at the end of both oxygen regimes (Table 4). In oxygenated water, concentrations of individual PLFAs, as well as the sum of these, were not correlated with extractable ^{203}Hg ($r < 0.75$, $P > 0.1$).

As in the water, the sum of the concentrations of the different PLFAs did not correlate with methyl ^{203}Hg or with extractable ^{203}Hg in the 0- to 1-cm sediment layers whereas individual PLFAs did, in this case 10Me16:0 and i15:0 (Table 4, Figs. 4A and 4B).

The first measurement of cobalamin (on day 23) revealed a significant positive correlation ($r = 0.88$, $P < 0.05$) between cobalamin and methyl ^{203}Hg in the anoxic water. In the oxygenated water, however, methyl ^{203}Hg seemed to vary inde-

© 1996 NRC Canada

Fig. 4. The concentration of methyl ^{203}Hg versus (A) the sum of the concentrations of all identified PLFAs in the 0- to 1-cm sediment layer (Table 5), and (B) the concentration of the PLFA 10Me16:0 in the 0- to 1-cm sediment layer ($r = 0.92$, $P < 0.0005$). Each symbol represents one microcosm. (One sample was analysed in each microcosm.)


A
Methyl 203Hg in sediment (log(cpm/g dry weight))
Total PLFA in sediment (log (nmol/g dry weight))
B
Methyl 203Hg in sediment (log (cpm/g dry weight))
10Me16:0 in sediment (log (nmol/g dry weight))

pendently of the cobalamin concentration ($r = -0.602$, $P > 0.25$). Later in the experiment, the positive correlation between methyl ^{203}Hg and cobalamin in the water of the anoxic microcosms disappeared (as measured on the last day of the first oxygen regime) and there was no such correlation after the change in the oxygen regime (Table 4). In the sediment, however, there was a significant positive correlation between cobalamin and methyl ^{203}Hg at the end of the experiment (Table 4, Fig. 5). Consequently, cobalamin was also significantly correlated with 10Me16:0 and i15:0 in the sediment ($r = 0.89$ ($P < 0.001$) and $r = 0.66$ ($P < 0.05$), respectively).

Sediment–water interactions

At the end of the experiment, extractable ^{203}Hg in the 0- to 1-cm sediment layer was found to be significantly correlated with extractable ^{203}Hg in the water, both for the oxygenated and the anoxic microcosms ($r = 0.99$, $P < 0.005$, and $r = 0.90$, $P < 0.05$). Furthermore, when all of the microcosms were included ($n = 10$), the highest concentration of extractable ^{203}Hg that was reached in the water during the anoxic phase was strongly correlated to the methyl ^{203}Hg in the sediment at the end of incubation (Fig. 6). The PLFAs i15:0 and 10Me16:0, as well as the cobalamin, found in the 0- to 1-cm sediment layers likewise showed significant positive correlations with the highest concentration of extractable ^{203}Hg in the water ($r = 0.74$, $P < 0.05$, $r = 0.91$, $P < 0.0005$, and $r = 0.91$, $P < 0.0005$).

Table 4. Correlation coefficients from linear log-log regressions between extractable ^{203}Hg and various compounds (PLFAs and cobalamin) in anaerobic water ($n = 5$) and in the 0- to 1-cm sediment layers ($n = 10$) of the microcosms.

	r		
	Anaerobic water		0- to 1-cm sediment
	Day 37[a]	Day 78[b]	layers (day 78)[b]
Total PLFA[c]	0.64	0.87	−0.03
i15:0	0.80	0.98**	0.83**
15:0	0.86	0.87	0.73*
Br17:0/Br17:1	0.84	0.98**	−0.05
10Me16:0	0.61	0.73	0.92***
i17:0	0.87	0.99**	0.42
18:1w7c	0.52	0.99***	−0.28
18:1w7t	0.90*	0.98**	0.35
Cobalamin	0.07	0.35	0.90***

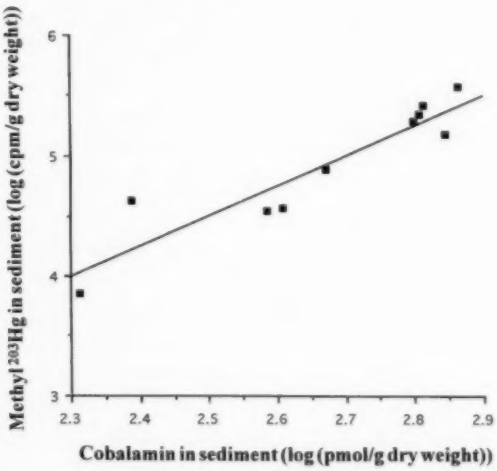
Note: Log-transformed concentrations were used in the calculations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. PLFAs listed in Table 5 but not presented here were not significantly correlated to extractable ^{203}Hg.

[a]The last day of the first oxygen regime.

[b]The last day of the second oxygen regime.

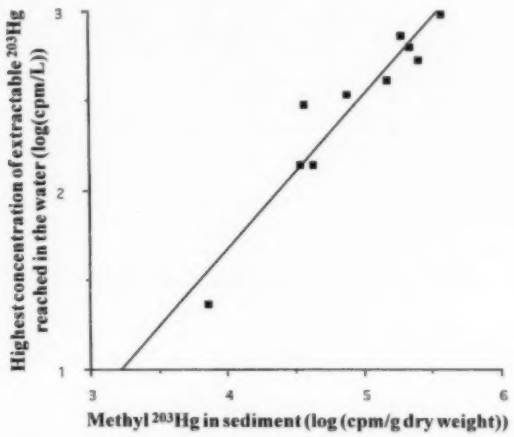
[c]The sum of the concentrations of all identified PLFAs listed in Table 5.

Fig. 5. The concentration of methyl ^{203}Hg versus the concentration of cobalamin in the 0- to 1-cm sediment layer ($r = 0.93$, $P < 0.0005$). Each symbol represents one microcosm. (One sample was analysed in each microcosm.)



© 1996 NRC Canada

Fig. 6. The highest concentration of extractable ^{203}Hg reached in the water during the anaerobic incubation versus the concentration of methyl ^{203}Hg in the 0- to 10-cm sediment layer at the end of the experiment ($r^2 = 0.91$, $P < 0.0005$). Each symbol represents one microcosm. (One water sample and one sediment sample were analysed in each microcosm.)



Comparison between fibre sediment and sediment without fibres

The microcosms containing fibreless sediment were among those that produced the least methyl ^{203}Hg (data not shown). Consequently, the difference in methyl ^{203}Hg concentration between oxygenated and anoxic water would have been larger if the microcosms with fibreless sediment had been excluded.

Since the fibreless sediment was low not only in methyl ^{203}Hg but also in 10Me16:0, i15:0, and cobalamin, exclusion of the fibreless sediment from the calculations would lower the significance of the positive correlation between the concentration of methyl ^{203}Hg and the concentration of these compounds in the sediment. However, when the values from the microcosms containing fibreless sediment were omitted from the regressions, 10Me16:0, i15:0, and cobalamin still showed significant correlation with methyl ^{203}Hg, with $r = 0.85$ ($P < 0.01$), $r = 0.87$ ($P < 0.005$), and $r = 0.96$ ($P < 0.0005$), respectively.

To elucidate differences in PLFA composition between the fibre sediment and the fibreless sediment (Table 5), and between the incubated sediment and the in situ sediment, a PCA of the PLFAs was performed. Included in the model were all of the 0- to 1-cm upper sediment layers from the microcosms ($n = 10$), five in situ sediment samples (0–1 cm) from the fibre deposit, and five in situ sediment samples (0–1 cm) from the deepest part of the lake (where the fibreless cores were taken). In a plot in which all of the sediment samples were projected onto a plane defined by the first and second principal components, four discrete clusters can be discerned, representing the eight microcosms containing fibre sediment, the two microcosms containing fibreless sediment, the in situ sediment from the fibre deposit, and the in situ sediment from the deepest part of the lake, respectively (Fig. 7A). Evidently, the composition of PLFAs in the sediment of the microcosms had changed with respect to the in situ composition. For example, comparing

Table 5. The phospholipid fatty acid composition of fibre sediment samples in situ (0–1 cm) and of fibre sediment (0–1 cm) incubated in microcosms.

PLFA[a]	In situ	Incubated
14:0	3.95±0.27	3.66±0.83
15:1a	0.34±0.03	0.11±0.20
Br15:0	0.30±0.02	0.10±0.15
i15:0	3.59±0.40	5.95±1.37
a15:0	4.83±0.48	6.48±0.43,
15:1b	0.11±0.05	0.00±0.00
15:1c	0.37±0.05	0.17±0.19
15:0	1.06±0.05	1.59±0.23
Br16:0	0.25±0.07	0.66±0.25
i16:0	0.79±0.06	1.35±0.21
16:1ω7c	29.42±2.45	18.83±2.86
16:1ω7t	4.07±0.37	4.12±0.78
16:1ω5	6.40±0.63	5.79±0.33
16:0	12.21±0.49	12.38±2.32
Br17:1/Br17:0	1.46±0.13	1.34±0.22
10Me16:0	1.10±0.14	2.35±1.10
i17:0	0.44±0.05	0.92±0.15
a17:0	0.44±0.05	1.73±0.22
17:1a	0.56±0.04	0.55±0.11
cy17:0	0.74±0.06	1.03±0.21
17:1b	0.49±0.04	0.74±0.10
17:0	0.47±0.03	0.70±0.07
18:3	0.60±0.15	0.29±0.06
18:2a	1.16±0.32	0.00±0.00
18:2b	1.01±0.12	0.19±0.31
18:1w9	3.87±0.35	5.05±0.52
18:1a	2.08±0.17	2.04±0.30
18:1w7c	13.72±1.80	14.85±1.26
18:1b	1.82±1.38	1.70±0.23
18:1w7t	0.42±0.01	0.55±0.18
19:1a	0.25±0.02	0.34±0.26
cy19:0	0.74±0.06	1.03±0.21
20:2	0.76±0.07	0.44±0.25

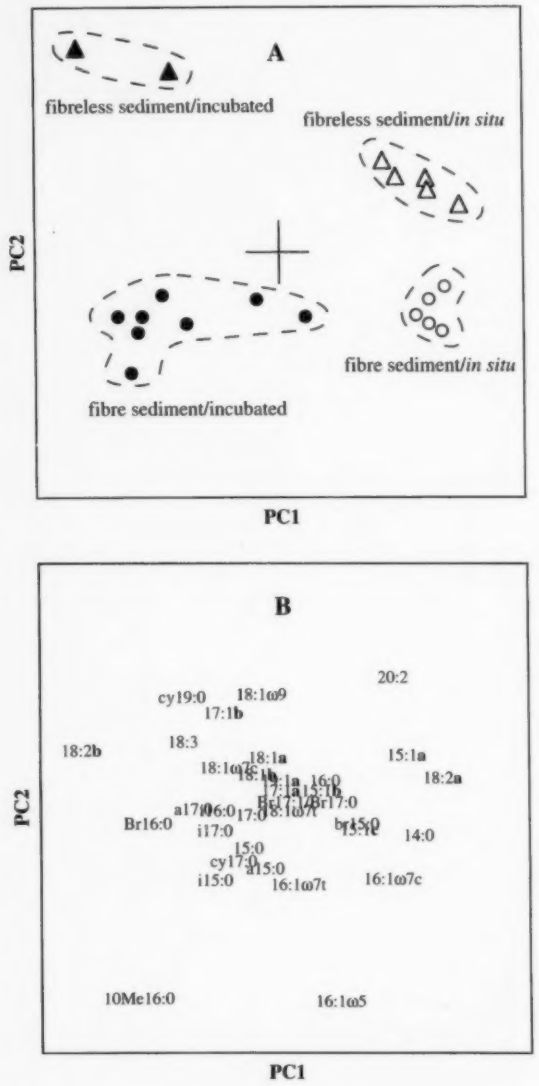
Note: Data are expressed as mole percent (relative contribution to the total PLFA content) and are given as the mean ± 1 standard deviation, on the basis of five in situ samples and eight incubated samples. The nomenclature of the PLFAs is explained in the text (Materials and methods).

[a]Fatty acids originating from phospholipids are presented in the order they eluted from the GC column. The fatty acid 18:0 was found in all samples but was omitted from the calculations because of high values obtained in blanks.

Figs. 7A and 7B, both showing the plane defined by the first and second principal components, it can be seen that the projection of 10Me16:0 is close to the projections of the incubated fibre sediment but far from the projections of the in situ fibre sediment. From this it follows that 10Me16:0 constituted a larger fraction of the total PLFA in the incubated fibre sediment than in the in situ fibre sediment (which also can be seen in Table 5). Since 16:1ω7c was close to the projections of the in situ fibre sediment but far from the projections of the incubated fibre sediment, the opposite was true for this PLFA. Furthermore, it is evident from Fig. 7A that the variation within the different clusters was larger for the clusters representing incubated sediment than for the clusters representing

© 1996 NRC Canada

Fig. 7. Plots based on a PCA model of the PLFA composition in sediment sampled in situ and in sediment sampled in the microcosms at the end of the incubation. (A) Projections of the different sediment samples on the plane defined by PC1 and PC2; 71% of the variation in PLFA composition is explained by this plane. (B) Projections of the PLFAs (those listed in Table 5) on the same plane. The cross indicates the origin. See text for further details.



in situ sediment, the distances between the objects being larger for the former clusters.

Discussion

Anoxic incubation of radiolabelled inorganic Hg(II) resulted in concentrations of methyl ^{203}Hg in the water 10–30 times higher than those produced under oxygenated incubation. This agrees well with earlier studies in which similar microcosms were used (Regnell and Tunlid 1991; Regnell 1994).

The difference in methyl ^{203}Hg concentrations between anoxic and oxygenated water did not seem to be related to demethylation, since the amount of volatilized ^{203}Hg was an order of magnitude less than the amount of methylated ^{203}Hg found in the microcosms. Furthermore, the decrease in methyl ^{203}Hg in the water following a change from anoxic to oxygenated conditions was not followed by a corresponding increase in volatilized ^{203}Hg. This confirmed results from an earlier study in which it was found that demethylation could not explain the difference in net production of methyl ^{203}Hg between anoxic and oxygenated microcosms (Regnell 1994). The underlying assumption, however, is that volatile Hg^0 is produced when methyl mercury is demethylated (Robinson and Tuovinen 1984).

Instead, high adsorption of methyl ^{203}Hg to solids under oxygenated conditions seemed to account for the disappearance of methyl ^{203}Hg in the water when the anoxic microcosms were oxygenated. This was indicated by the fact that a higher fraction of methyl ^{203}Hg was bound to particles in oxygenated water than in anoxic water. Thus, we believe that the sediment and possibly the walls of the microcosms acted as sinks for methyl ^{203}Hg when the microcosms were oxygenated. We hypothesize that the precipitation of ferric and manganese oxyhydroxides (or oxides) under oxic conditions caused the methyl ^{203}Hg to become particulate (Thanabalasingam and Pickering 1985; Dyrssen and Kremling 1990; Regnell 1995). It is also possible that the presence of sulphide under anoxic conditions increased the solubility of methyl mercury owing to the formation of the complex CH_3HgS^- (Wolfe et al. 1973, Dyrssen and Wedborg 1991). For total ^{203}Hg (essentially inorganic ^{203}Hg), however, the particulate fraction did not differ significantly between anoxic and oxygenated water. Under anoxic conditions, coprecipitation with FeS might have caused inorganic ^{203}Hg(II) to become particulate (Dyrssen and Kremling 1990).

No correlation was found between the concentration of methyl ^{203}Hg and the sum of the concentrations of the PLFAs in either the water column or the sediment. However, such a correlation was found for several of the individual fatty acids, including the methyl branch i15:0, 10Me16:0, and the monounsaturated 18:1ω7c/t. These correlations suggest that the structure of the bacterial community in both the water and the sediment was related to the degree of mercury methylation.

Iso- (and anteiso-) branched fatty acids are common in the lipids of Gram-positive bacteria but are rare in Gram-negative bacteria (O'Leary and Wilkinson 1988). The PLFA 10Me16:0 is characteristic of members of the genus of sulphate-reducing bacteria *Desulfobacter* (Taylor and Parkes 1983; Dowling et al. 1986), and this fatty acid has been used as a biomarker for *Desulfobacter* in aquatic sediments (Taylor and Parkes 1985; Parkes et al. 1993). Saturated 10Me-branched fatty acids are also produced in several actinomycete genera (Lechevalier 1977; Kroppenstedt 1985) but these bacteria produce mainly 10Me18:0 (Kroppenstedt and Kutzner 1976). Moreover, the actinomycete genera found in fresh water are mainly aerobes (Goodfellow and Williams 1983). Monounsaturated fatty acids with ω7 unsaturation indicate activity of the anaerobic desaturase pathway, which is found in all strict

© 1996 NRC Canada

anaerobic bacteria as well as in most Gram-negative aerobes (Fulco 1983).

When it was first reported that sulphate reducers were the main mercury methylators in aquatic sediments, a strain of *Desulfovibrio desulfuricans* was isolated that methylated mercury vigorously (Compeau and Bartha 1985). The monounsaturated methyl branched fatty acid i17:1ω7c is characteristic of *Desulfovibrio* (Edlund et al. 1985). It was not possible to quantify this fatty acid properly in our samples, but considering the retention time, we believe that this fatty acid was present in the peak designated Br17:1/Br17:0. The mass spectrum indicated that this peak contained both a saturated and an unsaturated methyl branched fatty acid. The concentration of Br17:1/Br17:0 was not correlated with the concentration of methyl ^{203}Hg in the sediment in our study. The fact that the concentration of methyl ^{203}Hg was strongly correlated with 10Me16:0 indicated that sulphate-reducing bacteria belonging to *Desulfobacter* rather than to *Desulfovibrio* were responsible for the mercury methylation in our study. Dowling et al. (1988) found that *Desulfobacter* contained relatively high amounts of i15:0. The positive correlation that we found between i15:0 and methyl ^{203}Hg in the sediment thus provided further evidence, albeit circumstantial, that *Desulfobacter* spp. methylated mercury.

It is tempting to infer a biochemical link between mercury methylation and the synthesis of 10Me16:0. This fatty acid, together with other 10Me-branched fatty acids, is biosynthesized by the addition of a methyl group transferred by methionine to the double bond in a straight-chain monounsaturated fatty acid (Asselineau 1966). Thus, considering that methionine is biosynthesized by the addition of a methyl group to homocysteine, the methyl group being transferred by a methyl cobalamin-containing enzyme (Foster et al. 1964), microorganisms synthesizing 10Me-branched fatty acids may have considerable needs for methyltransferase with methylcobalamin as a cofactor, which also might be capable of methylating mercury (Choi and Bartha 1993; Choi et al. 1994). Landner (1971) found evidence that mercury methylation can be viewed as an incorrect synthesis of methionine in the fungus *Neurospora crassa*, i.e., that inorganic Hg(II) was methylated instead of homocysteine. However, Landner did not mention the role of methyl cobalamin in this pathway, since *N. crassa* was not known to require vitamin B_{12}.

It can be argued that if we are right in our assumption that methyl mercury is produced in conjunction with the synthesis of 10Me16:0, the gross production of this fatty acid, rather than its level, should correlate with mercury methylation. However, since it can be assumed that the sediment in the different microcosms had approximately the same PLFA composition at the start of the experiment (except for the two microcosms containing sediment without fibres) (Table 5, Fig. 7), the actual levels of 10Me16:0 measured at the end of the incubation, which had increased significantly relative to the levels found in situ before incubation, probably reflected its gross production.

The correlation between methyl mercury, 10Me16:0, and cobalamin could be explained by mechanisms other than those suggested above. In fact, the strong positive correlation between methyl ^{203}Hg and cobalamin may indicate that acetogenic and (or) methanogenic bacteria were involved in mercury methylation, since these bacteria seem generally to contain levels of corrinoids orders of magnitude higher than those of sulphate-reducing bacteria (Inoue et al. 1992; Kräutler et al. 1988; Mazumder et al. 1987; Stupperich et al. 1988; Stupperich and Kräutler 1988). However, methanogenic bacteria seem to contain mostly pseudo-vitamin B_{12} and factor III (5-hydroxybenzimidazolyl-cobamide) (Mazumder et al. 1987; Stupperich and Kräutler 1988), which may not be detected by the B_{12} RIA kit used in our study.

Although extracts of methanogenic bacteria amended with B_{12} were shown to methylate inorganic Hg(II) (Wood et al. 1968), more recent studies have indicated that methanogenic bacteria are not involved in mercury methylation (McBride and Edwards 1977; Compeau and Bartha 1985, 1987).

To our knowledge, the ability of acetogenic bacteria to methylate mercury has never been investigated. An alternative interpretation of our results is that acetogenic bacteria methylated mercury, and that the correlation between 10Me16:0 and methyl ^{203}Hg was not a causal one. This could well have been the case considering the fact that acetate, which is produced by acetogenic bacteria, is the major carbon source for *Desulfobacter* (Postgate 1984). Thus, the growth of *Desulfobacter* spp., leading to an increase in 10Me16:0, should be expected to track that of acetogenic bacteria. Conversely, however, the fact that cobalamin was positively correlated with methyl ^{203}Hg could be the result of acetogenic bacteria, rich in cobalamin, stimulating the growth of *Desulfobacter* spp., which in turn methylated mercury. In neither case, although strongly correlated with methyl ^{203}Hg, was cobalamin necessarily limiting for the production of methyl mercury in this study. Unfortunately, we could find no information in the literature about the PLFA composition of acetogenic bacteria. However, 16S ribosomal RNA analysis has shown that acetogenic eubacteria belong to the Gram-positive spore-forming anaerobes, a group of bacteria not known to contain 10Me-branched fatty acids (Tanner et al. 1982; O'Leary and Wilkinson 1988).

In a previous study, using microcosms with sediment and water, we did not find any significant correlation between methyl ^{203}Hg and individual PLFAs or between methyl ^{203}Hg and cobalamin in the sediment (Regnell and Tunlid 1991). This might be explained by the fact that the sediment and water used in the previous study originated from a pristine lake, in which mercury methylation might be carried out by microbial consortia lacking signature PLFAs. However, the probability of finding such correlations was probably lower in that study than in the present study for several reasons. (*i*) The incubation time was shorter (24–41 days). Thus, most of the mercury methylation probably took place in the water and not in the sediment, since most of the added mercury was still in the water during the incubation. (*ii*) Four of the eight microcosms were kept oxygenated until the end of the experiment. The results from the present study indicate that individual PLFAs and cobalamin correlate much less with mercury methylation under oxic than under anoxic conditions. (*iii*) The entire sediment cores (homogenized) were used in the analyses, although it was probably only the uppermost layer that was involved in mercury methylation.

Certainly fibre sediment is an atypical freshwater sediment. It is thus possible that it is not representative of most freshwater sediments concerning the ability to methylate mercury and the mechanisms behind mercury methylation. Also note that the variation in the PLFA composition became higher in the

© 1996 NRC Canada

incubated sediment than it was initially in situ, which may have been due to a varying degree of disturbance of the sediment during sampling, and to the fact that the microbial flora had less time to reach equilibrium in the microcosms than in situ. Furthermore, the in situ microbial community might have responded in a different way to a rise in temperature and changing oxygen conditions than the microbial community in the microcosms did. Nevertheless, the high variation in PLFA composition in the sediment of the microcosms in fact improved our ability to study relationships between PLFA composition and mercury methylation.

Of the PLFAs found in anoxic water, only 18:1ω7t was significantly correlated with extractable ^{203}Hg at the end of the two oxygen regimes. This PLFA did not correlate at all with methyl ^{203}Hg or extractable ^{203}Hg in the sediment. Since the added ^{203}Hg, initially the water, steadily partitioned into the sediment, the sediment probably became an increasing source of methyl ^{203}Hg to the water. Thus, by the end of the first oxygen regime, conditions in the sediment rather than conditions in the water regulated the concentration of methyl ^{203}Hg in the water. This was indicated by the fact that there was a strong correlation between extractable ^{203}Hg in the sediment and in the anoxic water at the end of both oxygen regimes. We believe therefore that the correlation found between 18:1ω7t and extractable ^{203}Hg in anoxic water was not a causal one. This was probably also the case for Br17:0/Br17:1, i17:0, and 18:1ω7c at the end of the second oxygen regime.

It would be of great value if one could predict the capacity of a given substrate to methylate mercury. Our results indicate that analysing the composition of PLFAs could serve this purpose. Cobalamin might also be worthwhile to measure, although its relationship with mercury methylation seems far from clear. In theory, a model predicting mercury methylation should depend on parameters that adequately describe (*i*) the microbial community composition, (*ii*) the prevalence of biochemical pathways leading to mercury methylation, and (*iii*) the bioavailability of inorganic Hg(II). To predict net methylation, parameters associated with demethylation (e.g., resistance genes or their expression) may also have to be considered. An important point is that the level of methyl mercury in a substrate does not necessarily reflect the ability of that substrate to methylate mercury, since methyl mercury has a high degree of mobility in most substrates, and is likely to end up wherever the concentration of binding sites is high, e.g., in the aquatic biota.

Acknowledgements

This study was supported by the Swedish Environmental Protection Agency, Grycksbo Papyrus Ltd., and the Swedish Environmental Research Group. Professor Anders Södergren provided valuable comments on the manuscript. We also thank Åsa Frostegård and Dr. Erland Bååth for helpful discussions on multivariate analysis.

References

Asselineau, J. 1966. The bacterial lipids. Hermann, Paris. pp. 146–147.

Bertilsson, L., and Neujahr, H.Y. 1971. Methylation of mercury compounds by methylcobalamin. Biochemistry, **10**: 2805–2808.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911–917.

Bloom, N.S., and Effler, S.W. 1990. Seasonal variability in the mercury speciation of Onondaga Lake (New York). Water Air Soil Pollut. **53**: 251–265.

Choi, S.-C., and Bartha, R. 1993. Cobalamin-mediated mercury methylation by *Desulfovibrio desulfuricans* LS. Appl. Environ. Microbiol. **59**: 290–295.

Choi, S.-C., Chase, T., and Bartha, R. 1994. Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. Appl. Environ. Microbiol. **60**: 1342–1346.

Compeau, G., and Bartha, R. 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. Appl. Environ. Microbiol. **48**: 1203–1207.

Compeau, G., and Bartha, R. 1985. Sulphate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediments. Appl. Environ. Microbiol. **50**: 498–502.

Compeau, G., and Bartha, R. 1987. Effect of salinity on mercury-methylating activity of sulphate reducing bacteria in estuarine sediments. Appl. Environ. Microbiol. **53**: 261–265.

Craig, P.J., and Moreton, P.A. 1985. The role of speciation in mercury methylation in sediments and water. Environ. Pollut. Ser. B Chem. Phys. **10**: 141–158.

Dowling, N.J.E., Widdel, F., and White, D.C. 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. J. Gen. Microbiol. **132**: 1815–1825.

Dowling, N.J.E., Nichols, P.D., and White, D.C. 1988. Phospholipid fatty acid and infra-red spectroscopic analysis of a sulphate-reducing consortium. FEMS Microbiol. Ecol. **53**: 325–334.

Dyrssen, D., and Kremling, K. 1990. Increasing hydrogen sulfide concentration and trace metal behavior in the anoxic Baltic waters. Mar. Chem. **30**: 193–204.

Dyrssen, D., and Wedborg, M. 1991. The sulphur-mercury system in natural waters. Water Air Soil Pollut. **56**: 507–519.

Edlund, A., Nichols, P.D., Roffey, R., and White, D.C. 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. J. Lipid Res. **26**: 982–988.

Foster, M.A., Dilworth, M.J., and Woods, D.D. 1964. Cobalamin and the synthesis of methionine by *Esherichia coli*. Nature (London), **201**: 39–42.

Fulco, A.J. 1983. Fatty acid metabolism in bacteria. Prog. Lipid Res. **22**: 133–160.

Gilmour, C.G., and Henry, E.A. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. **71**: 131–169.

Gilmour, C.G., Henry, E.A., and Mitchell, R. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. **26**: 2281–2287.

Goodfellow, M., and Williams, S.T. 1983. Ecology of Actinomycetes. Annu. Rev. Microbiol. **37**: 189–216.

Guckert, J.B., and White, D.C. 1988. Evaluation of a hexane/isopropanol lipid solvent system for analysis of bacterial phospholipids and application to chloroform-soluble Nuclepore (polycarbonate) membranes with retained bacteria. J. Microbiol. Methods, **8**: 131–137.

Inoue, K., Kageyama, S., Miki, K., Morinaga, T., Kamagata, Y., Nakamura, K., and Mikami, E. 1992. Vitamin B_{12} production by *Acetobacterium* sp. and its tetrachloromethane-resistant mutants. J. Ferment. Bioeng. **73**: 76–78.

Kräutler, B., Kohler, H.-P.E., and Stupperich, E. 1988. 5′-Methylbenzimidazolyl-cobamides are the corrinoids from some sulphate-reducing and sulfur-metabolizing bacteria. Eur. J. Biochem. **176**: 461–469.

Kroppenstedt, R.M. 1985. Fatty acids and menaquinone analysis of actinomycetes and related organisms. *In* Chemical methods in bacterial systematics. *Edited by* M. Goodfellow and D.E. Minnikin. Academic Press Ltd., London. pp. 173–199.

© 1996 NRC Canada

Kroppenstedt, R.M., and Kutzne, H.J. 1976. Biochemical taxonomy of some problem actinomycetes. *In* Proceedings of the International Symposium on *Nocardia* and *Streptomyces*. Warsaw, Poland, October 4–8, 1976. *Edited by* M. Mordarski, W. Kurylowicz, and J. Jeljaszewicz. Gustav Fischer Verlag, Stuttgart. pp. 125–133.

Kvalheim, O.M., and Karstang, T.V. 1987. A general-purpose program for multivariate data analysis. Chemometrics Intelligent Lab. Syst. **2**: 235–237.

Landner, L. 1971. Biochemical model for the biological methylation of mercury suggested from methylation studies in vivo with *Neurospora crassa*. Nature (London), **230**: 452–454.

Lechevalier, M.P. 1977. Lipids in bacterial taxonomy: a taxnomist's view. Crit. Rev. Microbiol. **5**: 109–210.

Mazumder, T.K., Nishio, N., Fukuzaki, S., and Nagai, S. 1987. Production of extracellular methanol by *Methanosarcina barkeri*. Appl. Microbiol. Biotechnol. **26**: 511–516.

McBride, B.C., and Edwards, T.L. 1977. Role of the methanogenic bacteria in the alkylation of arsenic and mercury. *In* Biological Implications of Metals in the Environment: Proceedings of the 15th Annual Hanford Life Sciences Symposium, Richland, Washington, September 29–October 1, 1975. *Edited by* H. Drucker and R.E. Wildung. Technical Information Center and Energy Research and Development Administration, Springfield, Va. pp. 1–19.

Nichols, P.D., Guckert, J.B., and White, D.C. 1986. Determination of monounsaturated fatty acid double bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. J. Microbiol. Methods, **5**: 49–55.

O'Leary, W.M., and Wilkinson, S.G. 1988. Gram-positive bacteria. *In* Microbial lipids. Vol. 1. *Edited by* C. Ratledge and S.G. Wilkinson. Academic Press Ltd., London. pp. 117–185.

Oremland, R.S., Culbertson, C.W., and Winfrey, M.R. 1991. Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulphate reducers in oxidative demethylation. Appl. Environ. Microbiol. **57**: 130–137.

Osterdahl, B.-G., Janné, K., Johansson, E., and Johnsson, H. 1985. Determintion of vitamin B_{12} in gruel by a radioisotope dilution assay. Int. J. Vit. Nutr. Res. **56**: 95–99.

Ott, W.R. 1990. A physical explanation of the lognormality of pollutant concentrations. Air Waste, **40**: 1378–1383.

Parkes, R.J., Dowling, N.J.E., White, D.C., Herbert, R.A., and Gibson, G.R. 1993. Characterization of sulphate-reducing bacterial populations within marine and estuarine sediments with different rates of sulphate reduction. FEMS Microbiol. Ecol. **102**: 235–250.

Porter, K.G., and Feig, Y.S. 1980. Enhanced detection of bacteria in natural environments by fluorochrome staining of DNA. Limnol. Oceanogr. **25**: 948–951.

Postgate, J.R. 1984. The sulfate-reducing bacteria. 2nd ed. Cambridge University Press, Cambridge. pp. 67–68.

Ramlal, P.S., Rudd, J.W.M., and Hecky, R.E. 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. Appl. Environ. Microbiol. **51**: 110–114.

Regnell, O. 1994. The effect of pH and dissolved oxygen levels on methylation and partitioning of mercury in freshwater model systems. Environ. Pollut. **84**: 7–13.

Regnell, O. 1995. Methyl mercury in lakes: factors affecting its production and partitioning between water and sediment. Ph.D. thesis, Lund University, Lund, Sweden.

Regnell, O., and Tunlid, A. 1991. Laboratory study of chemical speciation of mercury in lake sediment and water under aerobic and anaerobic conditions. Appl. Environ. Microbiol. **57**: 789–795.

Robinson, J.B., and Tuovinen, O.H. 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses. Microbiol. Rev. **48**: 95–124.

Ryhage, R., and Stenhagen, E. 1960. Mass spectrometry in lipid research. J. Lipid Res. **1**: 361–390.

Sokal, R.S., and Rohlf, F.J. 1981. Biometry. 2nd ed. W.H. Freeman and Company, New York. pp. 427–428.

Spangler, W.J., Spigarelli, J.L., Rose, J.M., Flippin, R.S., and Miller, H.H. 1973. Degradation of methylmercury by bacteria isolated from environmental samples. Appl. Microbiol. **25**: 488–493.

Stupperich, E., and Kräutler, B. 1988. Pseudo vitamin B_{12} or 5-hydroxybenzimidazoloyl-cobamide are the corrinoids found in methanogenic bacteria. Arch. Microbiol. **149**: 268–271.

Stupperich, E., Eisinger, H.J., and Kräutler, B. 1988. Diversity of corrinoids in acetogenic bacteria. *P*-Cresolylcobamide from *Sporomusa ovata*, 5-methoxy-6-methylbenzimidazolylcobamide from *Clostridium formioaceticum* and vitamin B_{12} from *Acetobacterium woodi*. Eur. J. Biochem. **172**: 459–464.

Tanner, R.S., Stackebrandt, E., Fox, G.E., Gupta, R., Magrum, L.J., and Woese, C.R. 1982. A phylogenetic analysis of anaerobic eubacteria capable of synthesizing acetate from carbon dioxide. Curr. Microbiol. **7**: 127–132.

Taylor, J., and Parkes, R.J. 1983. The cellular fatty acids of the sulphate-reducing bacteria, *Desulfobacter* sp., *Desulfobulbus* sp. and *Desulfovibrio desulfuricans*. J. Gen. Microbiol. **129**: 3303–3309.

Taylor, J., and Parkes, R.J. 1985. Identifying different populations of sulphate-reducing bacteria within marine sediment systems, using fatty acid biomarkers. J. Gen. Microbiol. **131**: 631–642.

Thanabalasingam, P., and Pickering, W.F. 1985. Sorption of mercury (II) by manganese (IV) oxide. Environ. Pollut. Ser. B Chem. Phys. **10:** 115–128.

Tunlid, A., Hoitink, H.A.J., Low, C., and White, D.C. 1989. Characterization of bacteria that suppress Rhizoctonia damping-off in bark compost media by analysis of fatty acid biomarkers. Appl. Environ. Microbiol. **55**: 1368–1374.

White, D.C. 1988. Validation of quantitative analysis for microbial biomass, community structure, and metabolic activity. Ergeb. Limnol. **31**: 1–18.

White, D.C., Davis, W.M., Nickels, J.S., King, J.D., and Bobbie, R.J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia, **40**: 51–62.

Wolfe, N.L., Zepp, R.G., Gordon, J.A., and Baughman, G.L. 1973. Chemistry of methylmercurials in aqueous solution. Chemosphere, **4**: 147–152.

Wood, J.M., Kennedy, F.S., and Rosen, C.G. 1968. Synthesis of methyl-mercury compounds by extracts of a methanogenic bacterium. Nature (London), **220**: 173–174.

© 1996 NRC Canada

Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, *Salmo trutta*

Jörgen I. Johnsson, Erik Petersson, Elisabeth Jönsson, Björn Th. Björnsson, and Torbjörn Järvi

Abstract: In the hatchery environment, there is no selection by predators against risky foraging behaviour and excessive aggressiveness. Consequently, hatchery-reared trout should have a higher competitive ability and a less pronounced antipredator response than wild trout. Growth hormone increases the energy demand and thereby the feeding motivation of an animal. Therefore, growth hormone injections should reduce the antipredator response and promote competitive ability. Both hatchery selection and growth hormone injection consistently reduced antipredator behavioural responses in juvenile brown trout (*Salmo trutta*) in the presence of a trout predator. However, neither hatchery selection nor growth hormone had any effect on dominance or quantity of contested food consumed, whereas both hatchery selection and growth hormone injection promoted growth. RNA levels were increased by growth hormone, and hatchery-reared trout had lower RNA levels than wild trout. The presence of the predator increased RNA levels in the trout independent of strain and treatment. Our study suggests that antipredator behaviour, growth rate, and resource allocation patterns in brown trout change rapidly as a consequence of hatchery selection and that similar changes in antipredator behaviour can be induced by increasing GH levels. Thus, released or escaped hatchery-reared fish or fish with manipulated growth hormone levels with altered behaviour patterns may pose a potential threat to wild populations.

Résumé : Dans un milieu d'élevage, il n'y a pas de sélection par les prédateurs qui élimine le comportement d'alimentation à risque ni l'agressivité excessive. En conséquence, les truites d'élevage devraient présenter une compétitivité plus élevée, et une réaction antiprédateur moins prononcée que les truites sauvages. L'hormone de croissance augmente la demande d'énergie et donc la motivation à s'alimenter d'un animal. Les injections de cette hormone devraient donc réduire la réaction antiprédateur et promouvoir la compétitivité. De façon régulière, la sélection en élevage et l'injection d'hormone de croissance ont bien réduit les réactions comportementales antiprédateur chez les juvéniles de truite brune (*Salmo trutta*) en présence d'un prédateur. Toutefois, ni la sélection ni l'injection d'hormones n'avaient d'effet sur la dominance ou la quantité consommée de la nourriture contestée, tandis que les deux interventions avaient un effet favorable sur la croissance. Le taux d'ARN était accru par l'hormone, et les truites d'élevage présentaient des taux d'ARN plus faibles que les truites sauvages. La présence du prédateur faisait monter le taux d'ARN chez les truites, indépendamment de la souche et du traitement. Notre étude permet de penser que le comportement antiprédateur, le taux de croissance et le mode d'allocation des ressources chez les truites brunes changent rapidement par suite de la sélection piscicole, et que des changements similaires dans le comportement antiprédateur peuvent être induits par une augmentation du taux d'hormone de croissance. Les poissons libérés ou évadés des écloseries ou manipulés par injection d'hormone de croissance et présentant une modification comportementale peuvent donc constituer une menace pour les populations sauvages.
[Traduit par la Rédaction]

Introduction

In many organisms, survival and growth are correlated, at least during parts of their life. This correlation may be negative if increased feeding activity increases the risk of predation, or if fast growth occurs at the cost of investments in maintenance and repair (Sibly and Calow 1986). The trade-off between growth and survival is of central importance for understanding how predation and competition will affect dispersal, habitat choice, migration, and life history (Gilliam and Fraser 1987; Persson 1987, 1991; Werner and Hall 1988; Ludwig and Rowe 1990; Abrams 1991; Leonardsson 1991). Furthermore, it is evident from an increasing number of behavioural studies that animals possess the ability to adjust their foraging behaviour in response to variable predation risks (Lima and Dill 1990; Gilliam and Fraser 1987; Abrahams and Dill 1989).

Received July 12, 1995. Accepted February 15, 1996.
J12996

J.I. Johnsson.[1] Department of Zoology, Göteborg University, Medicinaregatan 18, S–413 90 Göteborg, Sweden.
E. Petersson. National Board of Fisheries, Fishery Research Station, Brobacken, S–810 70 Älvkarleby, Sweden.
E. Jönsson and B.Th. Björnsson. Department of Zoophysiology, Göteborg University, Medicinaregatan 18, S–413 90 Göteborg, Sweden.
T. Järvi. Institute of Freshwater Research, S–170 11 Drottningholm, Sweden, and Department of Zoology, Stockholm University, S–106 91 Stockholm, Sweden.

[1] Author to whom all correspondence should be addressed. e-mail: jorgen.johnsson@zool.gu.se

© 1996 NRC Canada

In fish, antipredator responses have a genetic basis but are also modified by learning (Magurran 1990; Järvi and Uglem 1993). Although temporal variations in the density and dispersal of food and predators may affect the optimal trade-off between foraging and predator avoidance (Holtby and Healey 1990), stabilizing selection against extreme behaviours in natural environments is expected. By contrast, selection against predator-vulnerable risky phenotypes may be relaxed in hatchery environments because the food supply is even and predictable, and natural predators are lacking (see Kohane and Parsons 1989). Further, high gain – high risk phenotypes may be favoured by direct or indirect (Swain and Riddell 1990) size selection in salmonid hatcheries. The latter hypothesis is supported by experiments in which larger rainbow trout (*Oncorhynchus mykiss*) foraged under higher predation risk than did smaller trout (Johnsson 1993). Indeed, studies by Johnsson and Abrahams (1991) suggest that hatchery-reared salmonids accept higher predation risks during foraging than wild salmonids.

It is less clear how domestication affects aggression. According to Ruzzante (1994), domestication can select either for increasing or decreasing aggression, depending on the amount of food provided in the hatchery, as well as its spatial and temporal distribution. Empirical studies on salmonids, however, generally suggest that aggression increases as a consequence of hatchery selection (e.g., Moyle 1969; Swain and Riddell 1990; Mesa 1991). As escalated fighting behaviour can increase predation risk by reducing vigilance (Jakobsson et al. 1995), aggression and antipredator responses are associated behaviours. Martel and Dill (1993) demonstrated that coho salmon (*Oncorhynchus kisutch*) were less aggressive when exposed to the odour of a bird predator.

Decisions that oppose feeding and predation risk may also be affected by the physiological state of an animal. For example, elevated hunger levels can increase risk proneness (Godin and Sproul 1988; Gotceitas and Godin 1991), aggression (Symons 1968; Dill et al. 1981), and competitive ability (Lindström et al. 1990; Andersson and Åhlund 1991; Lemel and Wallin 1993; Johnsson et al. 1996). Growth hormone (GH) stimulates tissue growth by increasing DNA synthesis and the rate of cell multiplication and differentiation (Isaksson et al. 1985), which may elevate hunger levels, thereby increasing the competitive ability of an animal. This is supported by data showing that appetite and competitive ability in rainbow trout are increased by GH injections (Johnsson and Björnsson 1994). GH may thus reduce antipredator responses by increasing feeding motivation, but this prediction is yet to be tested.

Altered growth and foraging behaviour may be explained ultimately through hatchery selection and proximately through changes in the endocrine regulation of growth. Furthermore, hatchery selection may alter endocrine growth regulation, which in turn may affect growth patterns and behaviour. The present study addresses the following hypotheses. (*i*) Domestication may promote risky foraging behaviour and excessive aggressiveness, since there is no selection by predators against these behaviours in hatcheries. (*ii*) GH increases the energy demand and thereby the feeding motivation of an animal, which may promote competitive ability and reduce antipredator responses during foraging.

These hypotheses generate four predictions. Hatchery-reared trout should have (1) higher competitive ability and (2) a less pronounced antipredator response than wild trout. GH-treated trout should have (3) higher competitive ability and (4) a less pronounced antipredator response than sham-treated trout (with normal GH levels).

We tested the above predictions in laboratory experiments by comparing growth patterns, competitive behaviour, and antipredator behaviour between GH- and sham-injected anadromous brown trout (*Salmo trutta*) of wild and hatchery-reared origin in the presence and absence of a brown trout predator.

Materials and methods

Experimental fish

Two strains of sea trout originating from the River Dalälven in Sweden occur in the river today. One strain was established in 1966 when a large number of fish were caught and used for artificial breeding and cultivation. The released offspring of these fish are marked by cutting the left pelvic fin so that they can be identified as adults to continue the breeding program. This strain is designated the sea-ranched strain. The other strain is a wild strain, consisting of all sea trout with intact pelvic fins, i.e., they had wild parents or, alternatively, sea-ranched parents spawning in the wild (for more details regarding the origin of these two stocks, see Petersson et al. (1995)). Between 1986 and 1992 the wild strain was also used for artificial breeding and the offspring were released without being marked. Nevertheless, the offspring of all of the unmarked sea trout studied will be referred to as the wild strain. In 1989, electrophoresis was carried out on the offspring of marked and unmarked parent fish (H. Jansson, Salmon Research Institute, Älvkarleby, Sweden, personal communication). Of eight loci investigated, four showed significant differences in gene frequencies between the two strains. (The fish were sampled from the offspring of 25–30 parent pairs).

The juvenile fish stocks used in this study were obtained in the following way: for each strain, the eggs of eight females were mixed and sperm from eight males were also mixed and used to fertilize the egg mix. Eggs were incubated and juveniles reared in the hatchery according to normal hatchery practice. The hatchery- and rearing-tanks were supplied with river water at ambient temperature and juveniles were given food pellets (Astra-Ewos) at a ratio of about 4% of body weight per day. Juveniles were reared in four tanks per strain and the experimental fish were sampled from these tanks in equal numbers. Initial weights and lengths were not different among treatment groups (Table 1). Wild trout, however, had a significantly higher condition factor than sea-ranched trout (see Table 1).

Experimental procedures

Treatment

The experiments were conducted in two similar series from 4 to 12 August and from 13 to 21 August. Each series consisted of two parts: a treatment period (days 1–4) and an observation period (days 5–9). Mean water temperature during the experiments was 12.3°C, and simulated natural photoperiod was maintained using electronic timers.

Starting on day 1 at 08:00, 72 fish from each strain were selected from the holding tanks. During anaesthesia (2-phenoxyethanol, 0.5 mL/L) body weight and fork length were recorded (see Table 1) and each fish was freeze branded dorsally (using a metal rod chilled in liquid nitrogen) with 1 of 12 different combinations of dots, allowing individuals to be distinguished during the experiments. Thereafter, half the fish received a dose of 5 mg ovine growth hormone (oGH)/g body weight through an intraperitoneal injection of 2 μL/g body weight using a 5- or 10-μL Hamilton syringe (Unimetrics, Shorewood, Ill.). The oGH was dissolved in 0.03 M NaCl, with 0.5% bovine serum albumin added, at a concentration of 2.5 mg oGH/mL

© 1996 NRC Canada

Table 1. Initial weight (W1), length (L1), and condition factor (CF1) in sham and GH-treated groups of juvenile brown trout of wild or sea-ranched origin.

Size and condition	Wild sham-injected	Wild GH-injected	Sea-ranched sham-injected	Sea-ranched GH-injected
W1	2.2±0.10	2.2±0.09	2.2±0.08	2.1±0.09
L1	6.0±0.09	6.0±0.08	6.0±0.07	6.0±0.07
CF1	0.98±0.01*a*	1.00±0.01*a*	0.96±0.01*b*	0.95±0.01*b*

Note: Values are given as the mean ± SE. Means followed by the same letter are not statistically different (Turkey's studentized range test, $\alpha = 0.05$).

and with pH adjusted to 9.5. The other half of the fish received a sham injection of the hormone vehicle only, yielding four subgroups of trout: (*i*) wild GH-treated (WGH); (*ii*) wild sham-treated (WS); (*iii*) sea-ranched, GH-treated (RGH); and (*iv*) sea-ranched, sham-treated (RS). The fish recovered quickly from the anaesthesia and behaved normally within a few minutes.

The fish were then separated into 12 groups of 12, each group consisting of three individuals from each subgroup, in stream tanks measuring 126 × 58 cm and with a water depth of 12 cm. Each tank was divided into a small and a large compartment, separated with a plastic net of sufficiently large mesh, 1.8 × 1.8 cm, that the juvenile trout could pass through it. The small compartment measured 42 × 58 cm and the large compartment 84 × 58 cm. The GH and sham injections were repeated during the 3 following days between 08:30 and 12:00, and on the last day of treatment, body weight and fork length were recorded. All volumes injected were based on individual initial weights. This GH-treatment protocol has previously been shown to generate significant changes in behaviour, growth, and osmoregulation in rainbow trout (Johnsson and Björnsson 1994).

The fish were not fed on the first treatment day. During the remaining treatment period, fish were fed once daily at 14:00, according to the following procedure: square-shaped pieces of frozen chironomid larvae were dropped by hand into a floating ring (10 cm diameter) situated in the middle of the small compartment of the stream tank. The purpose of the ring was to keep the food in place, thereby forcing the fish to enter the small compartment to get the food. However, some of the food (about 12%) drifted out of the small compartment and was eaten in the large compartment. Daily feeding rates were about 1.5% dry weight per fish wet weight. At 16:00 on the last treatment day, an adult brown trout (weight 54.3 ± 2.9 g (mean ± SE), fork length 17.4 ± 3.5 cm) was introduced to the small compartment of 6 of the 12 stream tanks. This trout was unable to pass through the net and thus remained in the small compartment throughout the experiment.

Observations

The behavioural observations started on the day after the last treatment day. These consisted of three main parts.

(*i*) At 08:00, the position and activity (holding high, holding low, or swimming) of each individual were recorded on a map over the stream tank (the bottom was divided into 78 numbered, equally sized squares). This recording was repeated after feeding and repeated again after aggressive interactions had been observed (see below). The recorded positions were used to measure the distance from each individual (nose) to the predator (nose) and to the floating ring (centre) and to assess whether the fish was positioned inside or outside the small compartment.

(*ii*) Between 09:00 and 12:00, food was provided according to the feeding procedure previously mentioned, and during the following 20 min we recorded in which order and the number of times each individual grasped a piece of food and in which compartment the fish was feeding.

(*iii*) Starting at 13:00, aggressive interactions between the individuals were observed during 15 min, and the winner and loser of each encounter was recorded. These observations were used to calculate a cardinal dominance rank for each individual according to Boyd and Silk (1983). Roughly, this dominance index is the natural logarithm of an individual's proportion of interactions won. This makes it possible to assess the magnitude or the significance of the difference in degree of dominance between individuals, rather than just measure the position of an individual in the hierarchy (ordinal dominance rank). The retained dominance index is a parametric value; thus, it is possible to use parametric statistical techniques to relate the rank of individuals to other variables of interest.

Level of aggressiveness was assessed in two ways: (*i*) as the total number of aggressive interactions and (*ii*) as the number of interactions won. The latter is probably a better estimate of an individual's aggressiveness, because the correlation between the number of interactions initiated and won is greater than that between the total number of interactions and those initiated (see Results). In the first case, an individual being conquered unusually often will be regarded as aggressive, which probably is incorrect.

The behavioural observations were recorded during 5 consecutive days. The final fork lengths and weights were then measured, and the fish were killed and frozen (–74°C) for RNA analysis.

RNA analysis

The amount of protein synthesis, as an indicator of current growth rate, can be estimated by measuring the amount of RNA in muscles (e.g., Bulow 1970; Houlihan et al. 1993; Jobling 1994). The RNA concentration in muscle was assessed in duplicate 50-mg samples according to the Schmidt–Thannhauser method (Munro and Fleck 1966; modified by Edsman et al. 1994), measuring the ultraviolet light absorbance at 260 nm on a Shimadzu UV-1201 spectrophotometer. The RNA concentration was expressed as milligrams per gram of the initial muscle sample.

Statistical analyses and treatment of variables

Many of the variables in this study were recorded as repeated measurements, e.g., an individual's position in relation to the food ring and the predator. In such cases, the median value for each individual was used as a general index. To estimate how the distance from each fish to the food ring varied over the experiment (DTRV), the difference between the third and first quartile for this distance was used. Specific growth rate in weight (SGRW) was calculated as (log(terminal body weight/initial body weight)) × 100/days. Relative food conversion efficiency was estimated as SGRW adjusted for number of food items (chironomid larvae) eaten per gram body weight.

All tests were two tailed and carried out using SAS statistical software (SAS Institute Inc. 1989). An analysis of variance of the different variables showed differences between the tanks used in the experiments, but the level of these differences was similar between the two trials if the effect of predator was kept constant. Therefore, variables were standardized with respect to tanks, whereafter replicates were pooled for further analysis. Since many of the continuous variables were correlated, analysis of covariance (ANCOVA) was frequently conducted, keeping the independent variable constant. In these cases, differences between groups were based on least squared means. For some variables, however, analysis of variance (ANOVA) was used. Variables not conforming to normal distribution were trans-

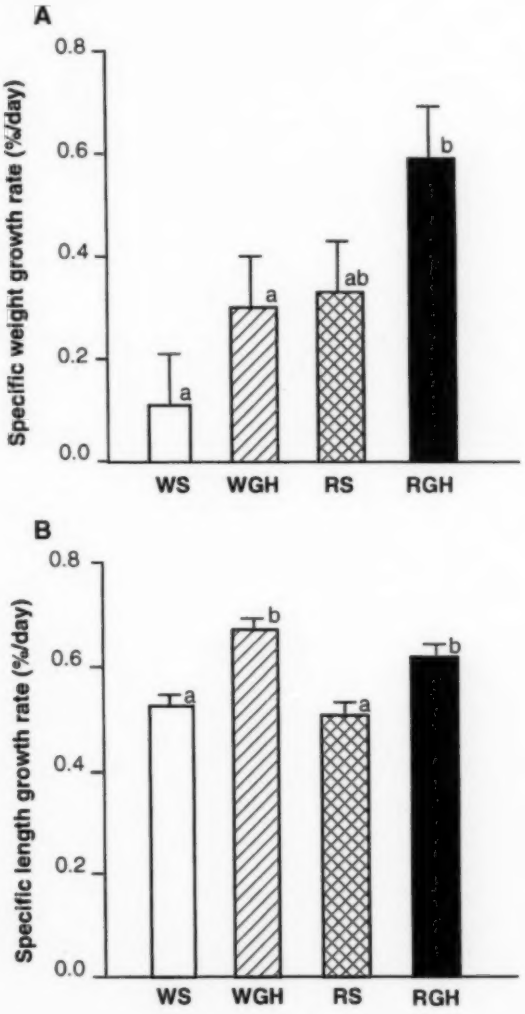
© 1996 NRC Canada

Table 2. Matrix of correlation coefficients between selected variables measured in juvenile brown trout.

	Inital length	Initial weight	SGRW	Food intake
SGRW	0.25*	0.13*	—	—
RNA level	−0.18**	−0.14*	0.17**	0.22*
Food intake	0.03	0.03	0.49***	—
Dominance	0.28***	0.27***	0.21***	0.27*
DTRV	−0.18*	−0.14**	−0.17**	−0.13*
Distance to food ring	−0.12*	−0.09	−0.12*	−0.21*
Distance to predator	−0.19*	−0.14	−0.16	−0.26*

Note: SGRW, specific growth rate in weight; DTRV, distance-to-ring variability.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Fig. 1. Mean specific weight (A) and length (B) growth rates for the four groups of juvenile brown trout. WS, wild, sham injected; WGH, wild, GH injected; RS, sea ranched, sham injected; RGH, sea ranched, GH injected. $n = 69–72$, $N = 279$. Error bars denote SEs of group means. Bars with the same letter are not significantly different.



formed using arcsine or log transformations. In one case (level of aggressiveness), normality could not be achieved through any transformation, so the nonparametric Kruskal–Wallis one-way ANOVA was used. In this case, differences between groups were analysed using Theil's slope estimator and distribution-free test for slope coefficient and Hollander's distribution-free test for the parallellism of two regression lines, which were calculated by hand following Hollander and Wolfe (1973).

Results

General correlations

In general, larger trout had lower RNA levels, higher SGRW, and higher dominance status than smaller fish (Table 2). Larger trout were also positioned closer to the predator, and the variability of their distance to the food ring (DTRV) was lower. Fast-growing trout (SGRW) had higher RNA levels, food intake, and dominance status than slower growing individuals; they were also closer to the predator and their DTRV was lower. A similar pattern was found with respect to food intake, which was expected because growth rate and food intake were positively correlated (Table 2).

Growth rate

Specific weight and length growth rates among treatment groups in the presence or absence of a predator were analysed by two-way ANCOVA, using dominance index as the covariate. The presence of a predator had no effect on weight ($F_{[1,270]} = 0.41$, $P > 0.5$) or length ($F_{[1,270]} = 1.31$, $P > 0.2$) growth rates, nor were there any predator–treatment interactions affecting weight or length growth rates ($F_{[3,270]} > 0.4$, $P > 0.7$ in both cases). As predator presence had no effect on growth, replicates with and without the predator were pooled for the growth analyses. Growth in weight was enhanced both by hatchery rearing and GH treatment (Fig. 1A). The RGH group grew fastest followed by the RS and WGH groups, which had similar growth rates, while the WS group grew slowest. Growth in length (Fig. 1B), however, was positively affected by GH, whereas hatchery rearing had no effect.

Food conversion

Relative food conversion efficiency (weight growth rate adjusted for food intake; see Materials and methods) was analysed using a two-way ANCOVA. There was a moderate difference among treatment groups ($F_{[3,276]} = 2.54$, $P = 0.06$) but no effect of predator presence or predator–treatment interaction ($P > 0.5$ in all cases). Further analysis of the difference

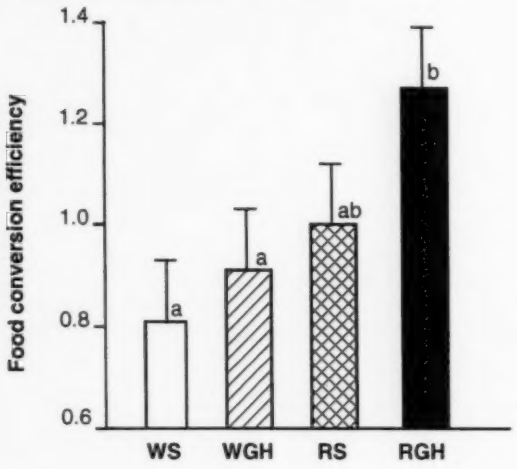
© 1996 NRC Canada

Table 3. Nonparametric regression slopes (all significant, $P < 0.001$) for total number of aggressive interactions and number of interactions won, dominance index being the independent variable in both cases.

	Wild sham-injected	Wild GH-injected	Sea-ranched sham-injected	Sea-ranched GH-injected
Interactions	1.05*a*	1.46*b*	1.00*a*	1.33*ab*
Interactions won	1.21*a*	1.42*b*	1.00*a*	1.00*ab*

Note: Groups of sham- or GH-treated brown trout of wild or sea-ranched origin are compared. Slope estimates followed by the same letter are not different at the 0.05 level, using a distribution-free method (see Methods).

Fig. 2. Mean food conversion efficiencies (specific weight growth rates with food intake kept constant) for the four groups of juvenile brown trout. WS, wild, sham injected; WGH, wild, GH injected; RS, sea ranched, sham injected; RGH, sea ranched, GH injected. n = 69–72, N = 279. Error bars denote SEs of group means. Bars with the same letter are not significantly different.



among treatment groups revealed that RGH trout had higher food conversion efficiency than the other groups (Fig. 2).

RNA levels

Variation in RNA levels was studied using two-way ANOVA. RNA levels were different among treatment groups ($F_{[3,272]}$ = 21.09, $P < 0.0001$). Further analysis showed that RNA levels were elevated by the GH treatment (Fig. 3) and were higher in wild trout than in sea-ranched trout. Trout that had been exposed to the predator had higher RNA levels than trout in tanks without a predator (1.5 vs. 1.4 mg/g body wet weight, respectively; $F_{[1,272]} = 2.54$, $P = 0.05$). There was no predator–treatment interaction ($P > 0.6$) for RNA levels.

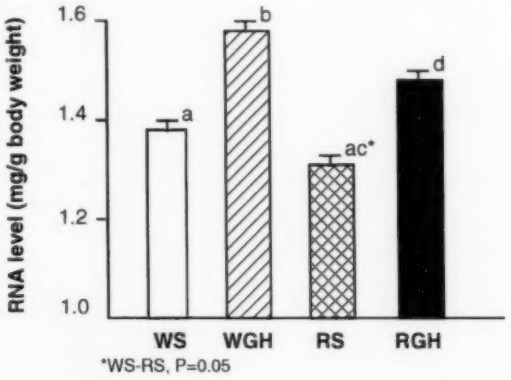
Food intake

Variation in food intake among treatment groups in the presence or absence of a predator was analysed by a two-way ANCOVA, using dominance index as the covariate. Neither treatment nor predator presence had any effect on food intake, and there was no predator–treatment interaction ($P > 0.3$ in all cases).

Dominance index

Dominance index was analysed using ANCOVA with initial

Fig. 3. Mean RNA levels for the four groups of juvenile brown trout. WS, wild, sham injected; WGH, wild, GH injected; RS, sea ranched, sham injected; RGH, sea ranched, GH injected. n = 69–72, N = 281. Error bars denote SEs of group means. Bars with the same letter are not significantly different.



weight kept constant. There were no differences among treatment groups, no effect of predator presence, and no predator–treatment interaction ($P > 0.6$ in all cases).

Level of aggressiveness

The total number of aggressive interactions or number of aggressive interactions won did not differ among groups (H = 1.46 and 3.29, respectively, both nonsignificant; Kruskal–Wallis one-way ANOVA). However, dominance index was correlated both with total number of interactions ($r_s = 0.448$, $P < 0.001$, $n = 287$) and number of won interactions ($r_s = 0.657$, $P < 0.001$, $n = 287$; Spearman rank correlation). Both variables increased significantly with dominance index, i.e., the dominant individuals were more aggressive than the subordinates (Table 3). This was even more clear if only interactions won were considered. Pairwise comparisons, using nonparametric methods, between slopes revealed that, for both variables, WGH had steeper slopes than WS and RS ($P < 0.05$), and there was a tendency for a difference between WGH and RGH as well, where only numbers of won interactions were considered. Otherwise no differences between slopes were found (Table 3).

Distance-to-ring variability

DTRV was analysed using two-way ANOVA. Predator presence increased DTRV (28.7 cm without predator and 42.4 cm with predator, respectively; $F_{[1,278]} = 11.5$, $P < 0.001$), but

© 1996 NRC Canada

Table 4. Position of juvenile brown trout in the stream tank in response to the presence or absence of a brown trout predator.

	Wild sham-injected		Wild GH-injected		Sea-ranched sham-injected		Sea-ranched GH-injected	
	Predator	No predator	Predator	No predator	Predator	No predator	Predator	No predator
Distance to food ring (cm)	80.5±7.1*	59.0±7.2	73.0±7.2	69.3±7.0	61.4±6.9	71.3±7.1	67.4±7.2	60.7±7.0
Time in small area (%)	32.8±6.7*	7.17±6.8	47.7±6.8	65.1±6.7	48.0±6.6*	70.9±6.7	52.6±6.8	49.9±6.7
Feeding in small area (%)	79.6±4.9*	92.9±3.3	87.3±4.0	93.5±3.6	86.5±3.8	93.7±3.1	85.0±4.6	88.2±4.3

Note: Values are given as the mean ± SE. Significant effects of predator presence within groups are indicated with an asterisk. Statistical tests are paired *t* tests on least square means ($\alpha = 0.05$), with dominance index held constant.

there were no differences among treatment groups and no predator–treatment interaction ($P > 0.1$ in both cases).

Antipredator behaviour

The median distance to the food ring and the proportion of time spent in the small compartment were analysed using multivariate ANCOVA. The presence of the predator did not significantly affect the distance to the food ring ($F_{[1,277]} = 1.92$, $P > 0.1$) but reduced the time spent in the small compartment ($F_{[1,277]} = 16.1$, $P < 0.0001$). For both of these variables there was a significant predator–treatment interaction ($F_{[3,277]} = 3$, $P < 0.05$ in both cases). Further analysis showed that these interactions were caused mainly by the WS trout group, which had a stronger antipredator response than the other groups (Table 4). In addition, the RS group responded more strongly to predator presence than did the RGH group. WS trout fed more frequently outside the small compartment (on drifting food particles, see Methods) when the predator was present, whereas the other groups were less sensitive to predator presence in this respect (Table 4). Overall, WS trout had the strongest antipredator response, WGH and RS trout had similar and weaker reponses, and RGH trout did not show any tendency to respond to predator presence. In total, 7 of 288 (or 2.4%) juveniles were killed and eaten by the predatory trout during the experiments. Of these, one was WS-, one was WGH-, two were RS-, and three were RGH-treated fish.

Discussion

The results of the present study are in agreement with prediction 2 that hatchery selection reduces antipredator responses in brown trout. For all three position measurements, WS trout responded more strongly to predator presence than did RS trout (Table 4). This is consistent with previous behavioural comparisons between wild steelhead trout and wild–domesticated hybrids (Johnsson and Abrahams 1991). The reduced antipredator responses in domesticated salmonids may be due to relaxed selection for trade-offs between foraging and predator avoidance in the hatchery (see Kohane and Parsons 1989). Another, not mutually exclusive, explanation is that high gain – high risk phenotypes may be favoured by direct or indirect size selection in the hatcheries. Indeed, in the present study, larger, faster growing individuals were positioned closer to the trout predator (see also Johnsson 1993).

Because domesticated (RS) trout spent less time in the small risky compartment when the predator was present, it appears that some antipredator response remains in the hatchery-reared strain but it is less pronounced than in wild trout (Table 4). Thus, WS trout ate more chironomids (20%) outside the small area when a predator was present than absent (7%), whereas the other groups showed no significant response to the predator in this respect (Table 4). Consistently, the predatory trout killed more sea-ranched trout (five) than wild fish (two). Previous studies have documented lower reproductive success and higher mortality rates in the wild in offspring of hatchery salmonids compared with wild offspring (Reisenbichler and McIntyre 1977; Dwyer and Piper 1984; Chilcote et al. 1986; Lachance and Magnan 1990). Furthermore, recent experiments indicate that hatchery-selected steelhead trout are more susceptible to prickly sculpin (*Cottus asper*) predation than are wild steelhead (Berejikian 1995). Together these findings suggest that the behavioural changes induced by hatchery selection impair the fitness of hatchery-produced salmonids released in the wild.

Like hatchery rearing, GH treatment reduced the antipredator response in brown trout (Table 4), which is consistent with our prediction 4. In light of this, there are potential risks associated with GH manipulations intended to improve growth performance in aquaculture (Agellon et al. 1988). For example, altered GH gene expression by transgenesis (Devlin et al. 1994) may affect gene pools of wild fish in the case of interbreeding or behavioural interactions between escaped GH-manipulated and wild salmonids. Increased exposure to predators, as a consequence of increased feeding activity in fish with elevated GH levels, may be one of the factors that limits the endogenous GH production in fish in natural waters (Sibly and Calow 1986), despite its growth-promoting effects (McLean and Donaldson 1993).

Prediction 3 was that GH increases competitive ability in brown trout. However, GH had no effect on dominance index or intake of contested food items in the present study, although it previously has been shown to increase competitive ability in rainbow trout (Johnsson and Björnsson 1994). Analyses of aggression levels revealed a somewhat different picture. There was no difference in level of aggressiveness between groups. However, WGH fish had a steeper slope (aggression versus dominance index) than RS and WS fish, and a similar tendency was found when they were compared with RGH fish (Table 3). This indicates that the wild trout responded more strongly than the sea-ranched trout to the GH treatment. In wild fish, GH increased the level of aggressiveness but only in dominant fish. The level of aggressive interactions was low, and the dominant fish were responsible for the majority of aggressive interactions observed. Most subordinate fish were not observed being involved in any interactions at all. Thus, it is possible that GH can amplify innate tendencies for aggressive behaviour but cannot alter the behaviour of nonaggressive phenotypes.

As for GH, no effect of hatchery rearing on dominance index or intake of contested food could be shown (prediction 1). Furthermore, aggression levels appeared to be unaffected

© 1996 NRC Canada

by domestication. This is not consistent with previous studies (Moyle 1969; Swain and Riddell 1990; Mesa 1991) showing that hatchery salmonids are more aggressive than their wild competitors. Experiments on other fish species (reviewed by Ruzzante 1994), however, suggest that aggression can sometimes decrease during domestication. These variable effects of domestication on dominance and aggression may be related to life-history differences between the different species studied. Furthermore, they may reflect rearing conditions that differ in how they select for aggressive interactions. Both the amount of food provided (Ruzzante 1994) and its spatial and temporal distribution (see Grant 1993 for a review) may affect the direction and intensity of selection for agonistic behaviour.

The present study suggests that hatchery selection and GH have similar weight growth promoting effects (Fig. 1A). This is consistent with previous studies in which growth rate in salmonids increases as a consequence of domestication (e.g., Hershberger et al. 1990; Johnsson et al. 1993) and GH administration (Donaldson et al. 1979; Johnsson and Björnsson 1994). As there was no effect of GH or hatchery selection on food intake, the weight growth differentiation among the four trout groups appears to be caused mainly by differences in food conversion efficiency (Markert et al. 1977; Johnsson and Björnsson 1994). Furthermore, GH affected growth in length of both wild and hatchery trout positively, which is consistent with previous work on salmonids (Donaldson et al. 1979). Hatchery selection, in contrast, had no effect on length growth, suggesting that hatchery trout stored surplus food mainly as fat. Wild fish had significantly higher RNA levels than hatchery trout, suggesting a higher rate of protein synthesis (Houlihan 1991; Jobling 1994). Thus, hatchery selection not only affects growth rate and antipredator behaviour, but also seems to alter resource allocation and metabolic patterns.

We also found that trout in tanks with a predator had significantly higher RNA levels than fish in predator-free tanks. One possible explanation for this novel finding is that the detection of a predator is genetically preprogrammed to induce an increase in the rate of protein synthesis in the juvenile trout. Because mortality, particularly during early life, is an inverse function of size (Ricker 1979; Hargreaves and Brasseur 1986), the purpose would be to increase growth rate and quickly bring fish to a size where they are less vulnerable to predation (Werner et al. 1983; Brönmark and Miner 1992). Indeed, the mean size (~1 g) of the seven trout killed by the predator in the present study was considerably below the overall mean size (~2 g) of the experimental fish.

To summarize, both GH treatment and hatchery rearing reduce antipredator responses and increase growth rate and food conversion in weight in brown trout. In these respects the results are consistent with our predictions, suggesting that a GH-treated wild trout will behave and grow similarly to a hatchery-reared trout. However, all actions of domestication and GH are not similar. RNA levels (and thus protein synthesis) are increased by GH administration, whereas hatchery selection appears to reduce RNA levels. This indicates that hatchery-reared fish allocate more resources to fat storage than wild trout, and it may also explain why hatchery trout grow faster than wild trout by weight but not by length. Dominance index and food intake were not affected by hatchery selection or GH treatment, and no main effects on aggression were found, although GH increased aggression in dominant wild fish.

The reduced antipredator responses, as a consequence of domestication and GH, may reduce survival in natural waters. For GH this may induce a negative feedback loop, limiting the endogenous production of GH in wild salmonids. In the hatchery, however, selective predation is not acting on the juvenile freshwater stage, so hatchery selection will continue to reduce antipredator responses, until the selective regime of the hatchery environment is changed, or until the genetic variation for antipredator behaviour ceases. Our results lead to the hypothesis that hatchery selection, which directly or indirectly selects for higher growth rate, is in fact selecting for individuals with more active endocrine growth regulatory components, e.g., higher GH secretion rate or higher GH receptor densities, which can be mimicked and (or) enhanced by GH treatment.

Natural selection constrains individual physiology and behaviour of animals in the wild within limits outside of which their fitness will decline. These constraints may be lifted either by genetic or physiological manipulations, as demonstrated in the present study, resulting in substantial changes in physiology and behaviour. For natural populations, the consequences of such manipulations are often unpredictable. Lessons, such as the catastrophic ongoing decline of Pacific salmonid populations, partly because of indiscriminate hatchery production of smolts (Waples 1991; Meffe 1992), should teach us not to overestimate the ability of technology to compensate for losses of natural resources.

Acknowledgments

We thank Birgitta Ekstrand for carrying out the RNA analyses, Anna-Carin Löf for excellent assistance during the experiment, and the staff at the Fishery Research Station in Älvkarleby for taking care of the fish. Ovine GH, lot No. AFP–9220A, was kindly supplied by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture. This work was supported by grants to J.I.J. (40.0357/93), B.Th.B. (41.0301/91), and T.J. (40.0006/91) from the Swedish Council for Forestry and Agricultural Research. E.P. and T.J. were also supported by the Swedish National Board of Fisheries.

References

Abrahams, M.V., and Dill, L.M. 1989. A determination of the energetic equivalence of the risk of predation. Ecology, **70**: 999–1007.

Abrams, P.A. 1991. Life history and the relationship between food availability and foraging effort. Ecology, **72**: 1242–1252.

Agellon, L.B., Emery, C.J., Jones, J.M., Davies, S.L., Dingle, A.D., and Chen, T.T. 1988. Promotion of rapid growth of rainbow trout (*Salmo gairdneri*) by a recombinant fish growth hormone. Can. J. Fish. Aquat. Sci. **45**: 146–151.

Andersson, S., and Åhlund, M. 1991. Hunger affects dominance among strangers in house sparrows. Anim. Behav. **41**: 895–897.

Berejikian, B.A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Can. J. Fish. Aquat. Sci. **52**: 2476–2482.

Boyd, R., and Silk, J.B. 1983. A method for assigning cardinal dominance ranks. Anim. Behav. **31**: 45–58.

Brönmark, C., and Miner, J.G. 1992. Predator-induced phenotypical

© 1996 NRC Canada

change in body morphology in crucian carp. Science (Washington, D.C.), **258**: 1348–1350.

Bulow, F.J. 1970. RNA–DNA ratios as indicators of recent growth rates of a fish. J. Fish. Res. Board Can. **27**: 2343–2349.

Chilcote, M.W., Leider, S.A., and Loch, J.J. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Trans. Am. Fish. Soc. **115**: 726–735.

Devlin, R.H., Yesaki, T.Y., Biagi, C.A., and Donaldson, E.M. 1994. Extraordinary salmon growth. Nature (London), **371**: 209–210.

Dill, L.M., Ydenberg, R.C., and Fraser, A.H.G. 1981. Food abundance and territory size in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. **59**: 1801–1809.

Donaldson, E.M., Fagerlund, U.H., Higgs, D.A., and McBride, J.R. 1979. Hormonal enhancement of growth in fish. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, J.D. Randall, and J.R. Brett. Academic Press, New York. pp. 455–597.

Dwyer, W.P., and Piper, G.P. 1984. Three-year hatchery and field evaluation of four strains of rainbow trout. N. Am. J. Fish. Manage. **4**: 216–221.

Edsman, L., Järvi, T., and Niejahr, B. 1994. The RNA concentration as an index of current growth rate in juvenile signal crayfish, *Pacifastacus leniusculus*. Nord. J. Freshwater Res. **69**: 149–152.

Gilliam, J.F., and Fraser, D.F. 1987. Habitat selection under predation hazard: test of a model with foraging minnows. Ecology, **68**: 1856–1862.

Godin, J.G.J., and Sproul, C.D. 1988. Risk taking in parasitized sticklebacks under threats of predation: effects of energetic need and food availability. Can. J. Zool. **66**: 2360–2367.

Gotceitas, V., and Godin, J.G.J. 1991. Foraging under the risk of predation in juvenile Atlantic salmon (*Salmo salar* L.): effects of social status and hunger. Behav. Ecol. Sociobiol. **29**: 255–261.

Grant, J.W.A. 1993. Whether or not to defend? The influence of resource distribution. Mar. Behav. Physiol. **23**: 137–153.

Hargreaves, N.B., and Brasseur, R.J. 1986. Size selectivity of coho (*Oncorhynchus kisutch*) preying on juvenile chum salmon (*O. keta*). Can. J. Fish. Aquat. Sci. **43**: 581–586.

Hershberger, W.K., Myers, J.M., Iwamoto, R.N., McAuley, W.C., and Saxton, A.M. 1990. Genetic changes in the growth of coho salmon (*Oncorhynchus kisutch*) in marine net-pens, produced by ten years of selection. Aquaculture, **85**: 187–197.

Hollander, M., and Wolfe, D.A. 1973. Nonparametric statistical methods. John Wiley & Sons, New York.

Holtby, L.B., and Healey, M.C. 1990. Sex-specific life history tactics and risk-taking in coho salmon. Ecology, **71**: 678–690.

Houlihan, D.F. 1991. Protein turnover in ectotherms and its relationship to energetics. Adv. Comp. Environ. Physiol. **5**: 1–43.

Houlihan, D.F., Mathers, E.M., and Foster, A. 1993. Biochemical correlates of growth rate in fish. *In* Fish ecophysiology. *Edited by* J.C. Rankin and B.J. Jensen. Chapman & Hall, London. pp. 45–71.

Isaksson, O.G.P., Edén, S., and Jansson, J.-O. 1985. Model of action of pituitary growth hormone on target cells. Annu. Rev. Physiol. **47**: 483–499.

Jakobsson, S., Brick, O., and Kullberg, C. 1995. Escalated fighting behaviour incurs increased predation risk. Anim. Behav. **49**: 235–239.

Järvi, T., and Uglem, I. 1993. Predator training improves behaviour of hatchery reared Atlantic salmon (*Salmo salar*) smolt. Nord. J. Freshwater Res. **68**: 63–71.

Jobling, M. 1994. Fish bioenergetics. Chapman & Hall, London.

Johnsson, J.I. 1993. Big and brave: size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. **45**: 1219–1225.

Johnsson, J.I., and Abrahams, M.V. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Can. J. Fish. Aquat. Sci. **48**: 243–247.

Johnsson, J.I., and Björnsson, B.Th. 1994. Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. **48**: 177–186.

Johnsson, J.I., Clarke, W.C., and Withler, R.E. 1993. Hybridization with domesticated rainbow trout (*Oncorhynchus mykiss*) reduces seasonal variation in growth of steelhead trout (*O. mykiss*). Can. J. Fish. Aquat. Sci. **50**: 480–487.

Johnsson, J.I., Jönsson, E., and Björnsson, B.Th. 1996. Dominance, nutritional state and growth hormone levels in rainbow trout (*Oncorhynchus mykiss*). Horm. Behav. **30**: 13–21.

Kohane, M.J., and Parsons, P.A. 1989. Domestication: evolutionary change under stress. Evol. Biol. **23**: 31–48.

Lachance, S., and Magnan, P. 1990. Performance of domestic, hybrid, and wild strains of brook trout, *Salvelinus fontinalis*, after stocking: the impact of intra- and interspecific competition. Can. J. Fish. Aquat. Sci. **47**: 2278–2284.

Lemel, J., and Wallin, K. 1993. Status signalling, motivational condition and dominance: an experimental study in the great tit, *Parus major* L. Anim. Behav. **45**: 549–558.

Leonardsson, K. 1991. Predicting risk-taking behaviour from life-history theory using static optimization technique. Oikos, **60**: 149–154.

Lima, S.L., and Dill, L.M. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**: 619–640.

Lindström, Å., Hasselquist, D., Bensch, S., and Grahn, M. 1990. Asymmetric contests over resources for survival and migration: a field experiment with bluethroats. Anim. Behav. **40**: 453–461.

Ludwig, D., and Rowe, L. 1990. Life-history strategies for energy gain and predator avoidance under time constraints. Am. Nat. **135**: 686–707.

Magurran, A.E. 1990. The inheritance and development of minnow anti-predator behaviour. Anim. Behav. **39**: 834–842.

Markert, J.R., Higgs, D.A., Dye, H.M., and MacQuarrie, D.W. 1977. Influence of bovine growth hormone on growth rate, appetite, and food conversion of yearling coho salmon (*Oncorhynchus kisutch*) fed two diets of different composition. Can. J. Zool. **55**: 74–83.

Martel, G., and Dill, L. 1993. Feeding and aggressive behaviours in juvenile coho salmon (*Oncorhynchus kisutch*) under chemically-mediated risk of predation. Behav. Ecol. Sociobiol. **32**: 365–370.

McLean, E., and Donaldson, E.M. 1993. The role of growth hormone in the growth of poikilotherms. *In* The endocrinology of growth, development and metabolism in vertebrates. *Edited by* M.D. Schreibman, C.G. Scanes, and P.K.T. Pang. Academic Press, New York. pp. 43–71.

Meffe, G.K. 1992. Techno-arrogance and halfway technologies: salmon hatcheries on the Pacific coast of North America. Conserv. Biol. **6**: 350–354.

Mesa, M.G. 1991. Variation in feeding, aggression, and position choice between hatchery and wild cutthroat trout in an artificial stream. Trans. Am. Fish. Soc. **120**: 723–727.

Moyle, P.B. 1969. Comparative behavior of young brook trout of domestic and wild origin. Prog. Fish.-Cult. **31**: 51–59.

Munro, H.N., and Fleck, A. 1966. The detection of nucleic acids. *In* Methods of biochemical analysis. Vol. 14. *Edited by* D. Glick. Interscience Publishers, New York. pp. 117–176.

Persson, L. 1987. The effects of resource availability and distribution on size class interaction in perch, *Perca fluviatilis*. Oikos, **48**: 148–160.

Persson, L. 1991. Behavioral response to predators reverses the outcome of competition between prey species. Behav. Ecol. Sociobiol. **28**: 101–105.

Petersson, E., Järvi, T., Steffner, N.G., and Ragnarsson, B. 1995. The effect of domestication on some life history traits of sea trout (*Salmo trutta*) and Atlantic salmon (*S. salar*). J. Fish Biol. In press.

Reisenbichler, R.R., and McIntyre, J.D. 1977. Genetic difference in growth and survival of juvenile hatchery and wild steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **34**: 123–128.

© 1996 NRC Canada

Ricker, W.E. 1979. Growth rates and models. *In* Fish physiology. Vol. 8. Bioenergetics and growth. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 677–743.

Ruzzante, D.E. 1994. Domestication effects on aggressive and schooling behavior in fish. Aquaculture, **120**: 1–24.

SAS Institute Inc. 1989. SAS/STAT guide for personal computers, version 6 edition. SAS Institute Inc, Cary, N.C.

Sibly, R.M., and Calow, P. 1986. Physiological ecology of animals. Blackwell, Oxford.

Swain, D.P., and Riddell, B.E. 1990. Variation in agonistic behavior between newly emerged juveniles from hatchery and wild populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. **47**: 566–571.

Symons, P.E.K. 1968. Increase in aggression and in strength of the social hierarchy among juvenile Atlantic salmon deprived of food. J. Fish. Res. Board Can. **25**: 2387–2401.

Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 124–133.

Werner, E.E., and Hall, D.J. 1988. Ontogenetic habitat shifts in bluegill: the foraging rate predation risk trade-off. Ecology, **69**: 1352–1366.

Werner, E.E., Gilliam, J.F., Hall, D.J., and Mittelbach, G.G. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology, **64**: 1540–1548.

© 1996 NRC Canada

The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef Creek, Washington

Thomas P. Quinn and N. Phil Peterson

Abstract: Wild juvenile coho salmon (*Oncorhynchus kisutch*) were individually marked in October 1990 and 1991 to evaluate the effects of habitat complexity and fish size on over-winter survival in Big Beef Creek, Washington. Habitat complexity was quantified for the habitat unit where the fish were collected and, in 1991, also for the 500-m reach downstream from the collection site. Survival, estimated from recovery of marked smolts at the stream's mouth, differed between years (25.4 and 46.2%) and also varied among habitat units and reaches within years. Survival was at most weakly correlated with complexity of the habitat units but was strongly correlated with the quantity of woody debris and density of habitat units in the 500-m reach, and distance from the estuary. Because distance covaried with habitat complexity, we could not ascertain which factor had the primary influence on survival. In addition, larger fish generally survived at a higher rate than smaller individuals. However, fish tagged above William Symington Lake were smaller in the fall but larger as smolts and had higher survival rates than those tagged below the lake. Taken together, these results reveal complex relationships between size, habitat, and growth that may affect over-winter survival and subsequent life-history events.

Résumé : Nous avons marqué individuellement des juvéniles de saumon coho (*Oncorhynchus kisutch*) sauvage, en octobre 1990 et 1991, pour évaluer les effets de la complexité de l'habitat et de la taille des poissons sur la survie hivernale dans le Big Beef Creek (Washington). Nous avons quantifié la complexité de l'habitat dans l'unité d'habitat où les poissons ont été prélevés et, en 1991, nous l'avons fait également dans le tronçon de 500 m situé en aval du lieu de prélèvement. La survie, estimée d'après la récupération de smolts marqués à l'embouchure du cours d'eau, différait d'une année à l'autre (25,4 et 46,2%) et variait aussi selon les unités d'habitat et les tronçons une même année. Le taux de survie était au moins faiblement corrélé à la complexité des unités d'habitat, et était fortement corrélé à la quantité de débris de bois et à la densité des unités d'habitat dans le tronçon de 500 m, et avec la distance par rapport à l'estuaire. Étant donné que la distance variait avec la complexité de l'habitat, nous n'avons pas pu établir quel facteur avait l'influence la plus forte sur la survie. De plus, les poissons de grande taille survivaient généralement en plus grande proportion que les individus de petite taille. Toutefois, les poissons marqués au-dessus du lac William Symington étaient plus petits à l'automne mais plus grands au stade du smolt, et présentaient des taux de survie plus élevés, que ceux marqués au-dessous du lac. Considérés ensemble, ces résultats révèlent des relations complexes entre la taille, l'habitat et la croissance, relations qui peuvent affecter la survie hivernale et les événements subséquents du cycle biologique.
[Traduit par la Rédaction]

Introduction

Studies of the ecology of juvenile salmonid fishes in freshwater often attempt to identify factors affecting growth, distribution, and survival of individuals and dynamics of the population. Such studies reveal interactions between egg production by the parental generation, density-dependent processes, and climatic factors resulting in variation in size and abundance of parr and smolts (e.g., Atlantic salmon, *Salmo salar*: Symons 1979; Baglinière et al. 1993; brown trout, *Salmo trutta*: Elliott 1993; steelhead trout, *Oncorhynchus mykiss*: Ward and Slaney 1993). Most juvenile coho salmon (*Oncorhynchus kisutch*) spend 1 or 2 years in freshwater prior to seaward migration and populations seem to be limited by the amount of freshwater rearing habitat available (e.g., Marshall and Britton 1990). This limitation results in part from their territorial behavior (Chapman 1962, 1966) and the stream's carrying capacity may be set during the summer low-flow period (Smoker 1955; Lestelle et al. 1993) by the amount of pool space (Nickelson et al. 1979). As a result of limited freshwater rearing space, the production of seaward migrating smolts is typically asymptotically related to the number of adults in the parent population. That is, once the stream's carrying capacity for juveniles is reached, an increase in the number of spawning adults will not increase the abundance of

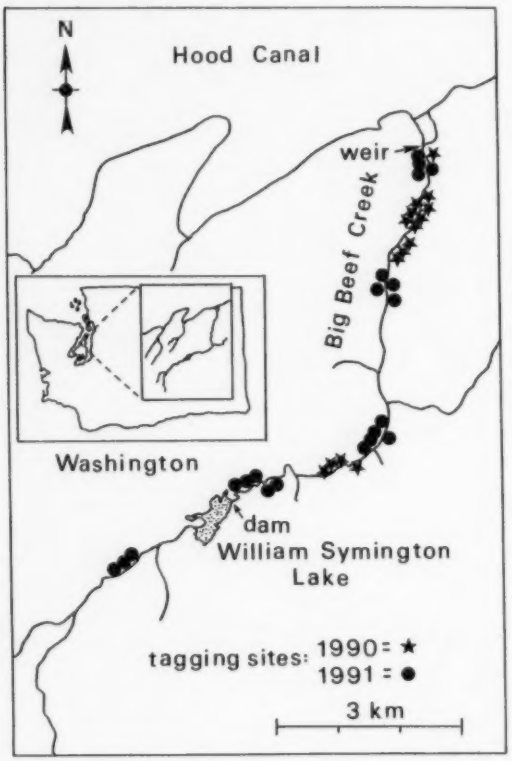
Received July 25, 1995. Accepted February 15, 1996.
J13012

T.P. Quinn[1] **and N.P. Peterson.**[2] School of Fisheries, University of Washington, Seattle, WA 98195, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: tquinn@fish.washington.edu
[2] Present address: Simpson Timber Co., P.O. Box 460, Shelton, WA 98584, U.S.A.

© 1996 NRC Canada

Fig. 1. Map of Big Beef Creek, showing approximate locations where juvenile coho salmon were tagged and released in October 1990 (stars) and 1991 (circles). The inset shows the study site's location on the Kitsap Peninsula, Washington.



smolts. Some of the variability around the asymptote may be related to interannual variation in summer flow conditions, but winter mortality can be severe and highly variable (Hartman et al. 1987) and may chiefly be a function of habitat rather than density-dependent factors (Murphy et al. 1984).

In coastal streams, winter freshets frequently cause stream bank erosion, streambed scour and fill, and rearrangements of accumulations of large woody debris (LWD). Juvenile coho salmon often move from summer nursery areas in streams to off-channel and floodplain habitats in winter (Cederholm and Scarlett 1982; Peterson 1982) but also overwinter in stream habitats (Scarlett and Cederholm 1984; Brown and Hartman 1988). During the winter, juvenile coho selectively inhabit deep pools with substantial accumulations of LWD (Bustard and Narver 1975; Murphy et al. 1986). Experiments by McMahon and Hartman (1989) and Shirvell (1990) indicated that coho salmon were attracted to the low velocities in the lee of woody obstructions. Thus, while off-channel habitat improves over-winter survival and growth for a segment of the population, the instream LWD that is a dominant feature of streams in forested watersheds (Bilby and Ward 1989, 1991) creates habitat conditions for most of the population. However, the association of coho salmon with LWD is not direct evidence that the debris reduces winter mortality.

Over broad expanses of the Pacific Northwest, removal of mature streamside forests during the initial logging and subsequent stream clean-out programs altered the amount and character of in-channel woody debris, resulting in simplified stream habitat (Bisson et al. 1987, 1992; Fausch and Northcote 1992). While loss of LWD from streams has been associated with reduced abundance of juvenile salmonids (Elliott 1986; Dolloff 1986), growth and production, particularly in summer, may also be strongly influenced by temperature (Holtby 1988), prey populations (Murphy et al. 1986; Bilby and Bisson 1987, 1992), fry density and freshets, and interactions among these factors (e.g., Scrivener and Andersen 1984). Holtby (1988) indicated that over-winter survival of underyearling coho salmon in Carnation Creek, British Columbia, could be explained in large part by the average fish size at the end of the summer. Estimating population-level changes that might result from habitat alteration may be difficult, in part because a small population may be composed of relatively large individuals at the end of summer that might experience above-average over-winter survival. Finally, marine survival of coho salmon may be related to both the smolt size and date of seaward migration (hatchery-produced salmon: Bilton et al. 1982; Morley et al. 1988; wild salmon: Thedinga and Koski 1984). Emigration date often varies with body size; larger and (or) older smolts often leave freshwater before smaller smolts (e.g., coho salmon: Seiler et al. 1981, 1984; Irvine and Ward 1989; Atlantic salmon: Jonsson et al. 1990; brown trout: Bohlin et al. 1993; sockeye salmon (*Oncorhynchus nerka*): Crawford and Cross 1992).

Woody material and the habitat complexity associated with it are thus interrelated with fish size in the ecology of juvenile stream-dwelling salmonids in complex ways (Bisson et al. 1987; Hartman et al. 1987). Studies of size, habitat, and survival have been largely limited to interannual comparisons (e.g., Hartman et al. 1987); data on individual fish are scarce and not always consistent with the predicted association between large size and survival (e.g., Brown 1985). However, as research on marine survival (e.g., Holtby et al. 1990; Henderson and Cass 1991) has revealed, the benefits of size may be more apparent within than among years. To better understand the interaction between summer growth and physical habitat as influences on over-winter survival, we measured and individually marked coho salmon from various habitats within a stream at the end of the summer and monitored their size and survival when they emigrated as smolts. Our specific hypotheses were (*i*) over-winter survival and size as smolts would be positively correlated with their size as underyearlings, (*ii*) the complexity of the habitat unit and stream reach where the fish were collected at the end of the summer would be positively correlated with survival, and (*iii*) larger smolts would leave earlier than smaller ones.

Methods

Study area

Big Beef Creek flows into Hood Canal from the Kitsap Peninsula, Washington (Fig. 1). It has a basin area of approximately 38 km^2 and contains 18 km of main stream channel, 8 km upstream and 10 km downstream of William Symington Lake, a 198-ha artificial impoundment constructed in 1965 (Williams 1970; Seiler et al. 1981). Fish pass over the 10-m dam and into the lake and streams above it

© 1996 NRC Canada

via a pool and weir fishway. Big Beef Creek's watershed is lower than 400 m elevation and the stream's dominant storm flows are from rains between November and March. A maximum flow of 21 m^3/s was recorded in 1971 and summer low flows near the mouth average 0.08 m^3/s. Above the lake the main stream channel is very flat (0.2% gradient) and in places is connected to extensive riparian wetlands. Below the lake the stream gradient lessens gradually from 1.5% in reaches below the dam to 0.5% near the mouth. Several small, relatively steep, intermittent tributaries enter Big Beef Creek in the 10 km below William Symington Lake.

In some of the lower stream reaches, coarse sediment generated during the basin's initial logging (as early as the late 1800s) has accumulated in the stream channel and is gradually being transported through the system (Madej 1975). Numerous old cedar stumps on the stream banks reflect the original conifer riparian forest that has regrown to red alder (*Alnus rubra*), vine maple (*Acer circinatum*), and bigleaf maple (*Acer macrophyllum*). Second-growth western hemlock (*Tsuga heterophylla*), western red cedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*) are also present.

Fish tagging and recovery

Juvenile coho salmon were captured in the 1st week of October 1990 and 1991 to represent the end of the summer growing period, prior to winter redistribution. Collections were made by stick seine from 15 habitat units in three stream reaches (all below the lake) in 1990 and 21 pools in five reaches in 1991, including sites above and below the lake. The fish were anesthetized with MS–222; fork length, weight, and home pool were recorded; and either a sequential coded wire (s-CW) or a passive integrated transponder (PIT) tag was implanted. They were allowed 30–60 min to recover and then returned to the pool where they had been collected. A 12-gauge hand-held hypodermic needle and syringe were used to implant the PIT tag into the body cavity (Peterson et al. 1994), and a 24-gauge needle and syringe were used to implant the s-CW tag into the fish's cranial cartilage. The adipose fin of each s-CW tagged fish was excised. A total of 717 and 954 fish were tagged in 1990 and 1991, respectively, evenly divided between s-CW and PIT tags.

Marked fish were recovered the following spring at a permanent weir at the mouth of the stream, operated from April 7 to June 14, 1991, and to June 15, 1992. All individuals were electronically scanned for the presence of PIT tags. The codes from individuals carrying PIT tags were recorded and the fish were weighed, measured, and released. All adipose-clipped fish were weighed, measured, and then sacrificed for subsequent s-CW tag extraction and reading. Survival was calculated as the proportion of fish tagged in the fall that were recovered in the spring. Individuals migrating downstream before or after the smolt sampling period would be classified as mortalities. The weir was operated during the period when almost all smolts emigrate (Seiler et al. 1984), but the survival estimates should be regarded as minima. We recovered seven age–2 smolts that had been marked in 1990 (three s-CW and four PIT tagged). Of the fish marked in 1991, no s-CW tagged individuals were recovered as age–2 smolts (they were not examined for PIT tags). Age–2 smolts were omitted from analysis because of their scarcity and because their longer residence period in the stream prevented comparison with age–1 smolts.

Habitat surveys

For purposes of comparing fish survival, we defined habitat complexity at two spatial scales: habitat unit and reach. A variety of features commonly used to describe streams in monitoring programs were measured for each habitat unit where the fish were collected. Habitat units were classified according to Bisson et al. (1982); most coho were taken from scour pools of various configurations, a plunge pool, and several glides. Bank-full channel width, low flow pool dimensions, and residual pool depth (Lisle 1987) were measured for each unit. An inventory of all the LWD in the study reaches was conducted and the volume of each piece of wood within the bank-full channel and below the bank-full depth (corresponding to influence zones 1 and 2 of Robison and Bestcha 1990) was measured using large calipers. LWD count and volume were related to reach length. In addition, LWD loading was computed as the number of pieces per square metre of bank-full channel.

In 1991, in addition to measurements of the habitat units, the 500-m reach of stream immediately downstream from each habitat unit was also surveyed for correlation with unit-specific survival rates. This definition of reach was based on the finding that most juvenile coho salmon in two small Olympic Peninsula streams spent the winter within 500 m downstream of their location at the onset of winter (Scarlett and Cederholm 1984). However, some of the lowermost reaches were less than 500 m above the estuary and were only measured to the counting fence at the upper edge of the estuary. Owing to this variation in reach length, we standardized our reporting of habitat reach characteristics to 100 m length of stream (e.g., LWD volume per 100 m). Some habitat units were less than 500 m apart, thus the 500-m reaches below habitat units sometimes overlapped. In addition to reporting habitat complexity as units per length of stream, we also used pool spacing, a more common metric used by fluvial geomorphologists to assess channel characteristics (e.g., Keller and Melhorn 1978; Montgomery et al. 1995). Pool spacing was calculated by dividing the length of the reach by the average channel width and the number of pools. We also evaluated the role of LWD in controlling habitat complexity (i.e., units per lineal distance and pool spacing). Over 3 km of stream was inventoried in reach-level surveys in 1991. No habitat surveys were done for the units above the lake because we assumed that the fish would overwinter in the lake or the numerous riverine wetlands that could not be clearly associated with one habitat unit or another.

Results

Overall estimated survival differed between years (25.4% in 1990–1991 and 46.2% in 1991–1992, $\chi^2 = 76.06$, $p < 0.01$; Table 1), but no differences in growth or survival could be attributed to effects of tag type in either year (Peterson et al. 1994). As a result of these findings and because the mean length and weight of fish differed between years at the time of marking (length: $t = 8.49$, $p < 0.01$; weight: $t = 14.82$, $p < 0.01$) and at the time of smolting (length: $t = 11.58$, $p < 0.01$; weight: $t = 9.80$, $p < 0.01$), we pooled data by tag type but analyzed size and habitat effects within each year separately. More of the fish tagged above the lake in 1991 survived than did those tagged below it (56.7 vs. 44.1%). However, the difference in survival between years was significant, even when the data from fish above the lake were removed and only lower reaches were compared (25.4% in 1990–1991 versus 42.1% in 1991–1992, $\chi^2 = 43.77$, $p < 0.001$).

Fish in larger size-classes showed a higher survival rate than smaller fish in 1990–1991 ($\chi^2 = 15.40$, $p < 0.01$) but not in 1991–1992 ($\chi^2 = 0.98$, $p = 0.91$; Fig. 2). Similarly, the average size at tagging between nonsurvivors and survivors differed in 1990–1991 but not in 1991–1992 (Table 1). Fish from a given area of the stream maintained their relative size ranking within the population; smaller fish at the end of the summer period were generally smaller smolts (Fig. 3). However, the fish tagged upstream from William Symington Lake were smaller in the fall than fish tagged below the lake but grew considerably more than their downstream-dwelling counterparts over the winter and were significantly larger as smolts (Table 2, Fig. 3).

Survival of fish from discrete habitat units varied in both

© 1996 NRC Canada

Table 1. Relationship between size in early October and over-winter survival of juvenile coho salmon in Big Beef Creek, Washington.

	Survivors			Nonsurvivors		
Season	*n*	Length (mm)	Weight (g)	*n*	Length (mm)	Weight (g)
1990–1991	182	75.7±7.8**	4.50±1.54**	535	73.6±7.4	4.05±1.40
1991–1992	441	76.5±8.1	5.12±1.64	513	76.0±7.8	5.03±1.50

Note: Values are given as the mean ± standard deviation. **, $p < 0.01$ (unpaired *t* test).

Table 2. Size of juvenile coho salmon captured above William Symington Lake (lake fish) and below the lake in Big Beef Creek (creek fish) in the fall when they were tagged and in the spring when the survivors were recovered as smolts.

	Lake fish			Creek fish		
Season	*n*	Length (mm)	Weight (g)	*n*	Length (mm)	Weight (g)
Fall	134	69.8±9.3	4.00±1.80	820	77.3±7.2	5.25±1.45
Spring	76	129.3±9.8	20.89±4.78	362	107.0±9.1	11.85±3.24

Note: Values are given as the mean ± standard deviation. All differences were significant at $p < 0.001$ on the basis of unpaired *t* tests.

Fig. 2. Over-winter survival of juvenile coho salmon of different sizes tagged at the end of summer in Big Beef Creek below William Symington Lake in 1990 (solid bars) and 1991 (hatched bars).

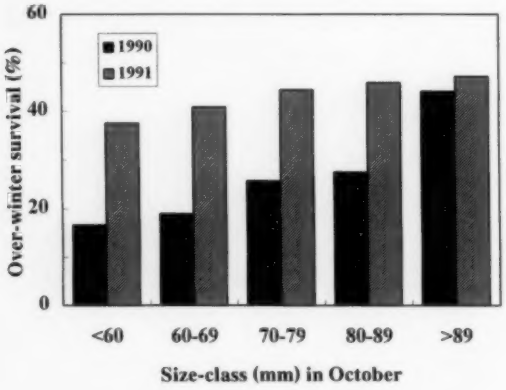
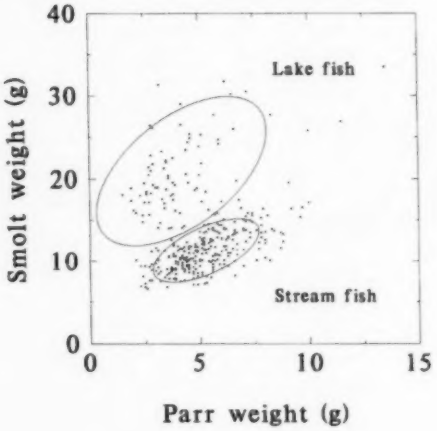


Fig. 3. Relationship between the weight of juvenile coho salmon in October 1991 and their weight as smolts in 1992. Individuals tagged above and below William Symington Lake are designated lake and stream fish, respectively. Ellipses are centered around the sample means and oriented along each axis of covariance.



years (1990–1991: $\chi^2 = 52.19$, df = 14, $p < 0.001$; 1991–1992: $\chi^2 = 51.55$, df = 20, $p < 0.001$). Habitat complexity, measured by variables in the individual habitat units, was poorly correlated with survival. No correlations were found with any of the variables tested in 1990–1991: depth and volume of the pool and volume and count of LWD. In 1991–1992 the volume and count of LWD in the unit showed weak positive correlations with survival (Table 3), but the other features of the units were not correlated with survival. However, survival was positively correlated with the number of habitat units below the tagging site, negatively correlated with pool spacing (Fig. 4), positively correlated with gradient and LWD volume and count, and negatively correlated with residual pool mean depth in the reach (Table 3). In both years, survival was positively correlated with distance from the estuary (Fig. 5). This relationship was calculated without the fish from the three habitat units above the lake that experienced relatively high survival rates (56.7% overall).

Stepwise multiple regression with number of habitat units per lineal distance, LWD frequency, and distance from the estuary as the independent variables indicated that survival was primarily controlled by distance ($r^2 = 0.77$). The addition of habitat unit frequency significantly increased explanatory power (multiple $r^2 = 0.84$), but LWD frequency made no additional statistical contribution to the model. Similar results were obtained when pool spacing was substituted for habitat units per lineal distance in the multiple regression (multiple $r^2 = 0.82$). Comparisons between habitat variables revealed significant ($p < 0.005$) correlations between habitat unit frequency and LWD frequency, volume, and loading, and distance from the estuary . Pool spacing was also significantly correlated ($p < 0.001$) with all measures of LWD abundance (e.g., Fig. 6) but not with distance from the estuary.

The median emigration dates were the 127th and 120th days of the year in 1991 and 1992 (i.e., 7 May and 30 April), respectively (Fig. 7). There was no clear relationship between smolt length and emigration date. However, the fish that had been tagged above the lake in 1991 were captured at the weir

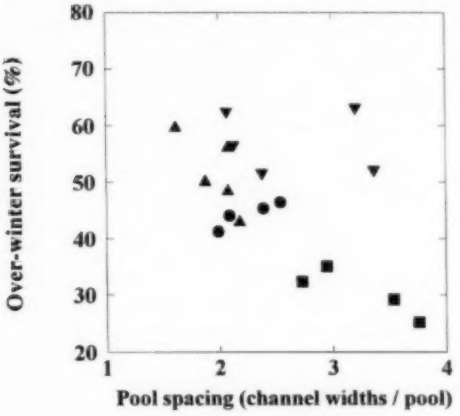
© 1996 NRC Canada

Table 3. Significant relationships between habitat features, measured at the scale of habitat unit or reach, and the over-winter survival of juvenile coho salmon tagged in Big Beef Creek in October 1990 (15 units) and 1991 (18 units).

Habitat feature	Scale	Year	r^2	p	Intercept	Slope
LWD count	Unit	1991	0.23	0.04	44.16	0.11
LWD volume	Unit	1991	0.23	0.04	44.00	0.23
LWD count	Reach	1991	0.61	0.001	30.50	0.34
LWD volume	Reach	1991	0.45	0.002	35.36	0.47
Residual depth	Reach	1991	0.34	0.01	70.62	−36.74
Gradient	Reach	1991	0.76	0.001	20.03	24.27
Pool spacing	Reach	1991	0.27	0.03	69.89	−9.21
No. of habitat units	Reach	1991	0.77	0.001	16.26	5.06
Distance from weir		1990	0.57	0.001	13.68	0.0035
		1991	0.77	0.001	32.87	0.0029

Note: Only units below William Symington Lake are included. LWD, large woody debris.

Fig. 4. Relationship between over-winter survival of juvenile coho salmon in Big Beef Creek and pool spacing in the 500-m reach below the unit where they were captured. Habitat units above William Symington Lake were not included in the analysis. Different symbols indicate distance from estuary (see Figs. 5 and 1).

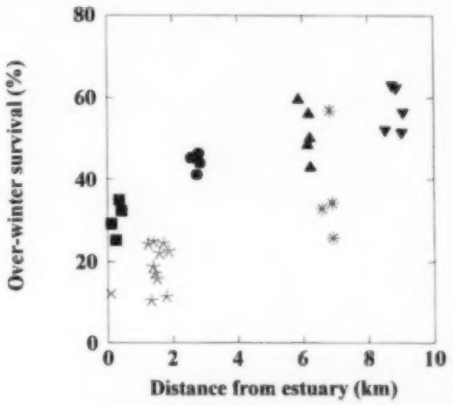


later than those tagged in the stream (day of the year 124.8 ± 6.4 (mean ± SD) vs. 120.8 ± 8.0, $t = 3.74$, $p < 0.01$).

Discussion

The salient findings of this study were the positive correlations between survival and body size, distance from the estuary, and reach-scale habitat complexity, and the difference in survival between years. Hartman et al. (1987) reported that the interannual variation in average survival of coho in Carnation Creek, British Columbia, was related to body size, but limited data on individual fish from an ephemeral swamp did not support this pattern (Brown 1985). Recent data on PIT-tagged chinook salmon in the Snake River indicated that the larger fish at the time of tagging in the summer were more likely to be detected as downstream migrating smolts the following spring (Walters et al. 1994; Achord et al. 1995*a*, 1995*b*). Our results indicated that the fish that were large in October maintained their size advantage to the smolt stage and had a higher probability of survival. The exceptions to this pattern were the fish tagged

Fig. 5. Relationship between over-winter survival of juvenile coho salmon and the distance from the estuary (i.e., the smolt counting weir) of the unit in which they were caught at the end of the summer. Units from the three reaches sampled in 1990 are indicated with a cross and five- and eight-pointed stars; the four reaches sampled in 1991 are indicated with solid symbols. Different symbols refer to groups of fish tagged at various distances form the estuary (see Fig. 1). Habitat units above William Symington Lake were not included in the analysis.



above William Symington Lake. They were relatively small at the end of the summer (perhaps because water temperatures tend to be cooler than those below the lake; Williams et al. 1968) but were larger than average as smolts, indicating a higher growth rate than that of fish tagged below the lake, and they also had high survival rates. We cannot be certain where any of the fish actually spent the winter, but such rapid growth would be consistent with pond or lake residence (Peterson 1982; Swales et al. 1988; Irvine and Ward 1989). However, they might also have overwintered in wetlands above the lake. Our results are consistent with those of Williams et al. (1968), who reported that coho sampled in the stream above the lake in September were smaller (mean length 54 mm) than those caught in the lake (91 mm) or in the stream below the lake (60–78 mm) but that by spring the fish in the upper stream had equaled the size of fish in the lower stream.

The causal link between fish size and survival is unclear.

© 1996 NRC Canada

Fig. 6. Relationship between pool spacing and large woody debris (LWD) frequency in study reaches of Big Beef Creek surveyed in 1991.

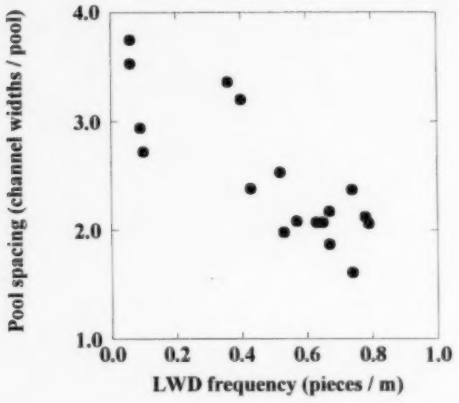
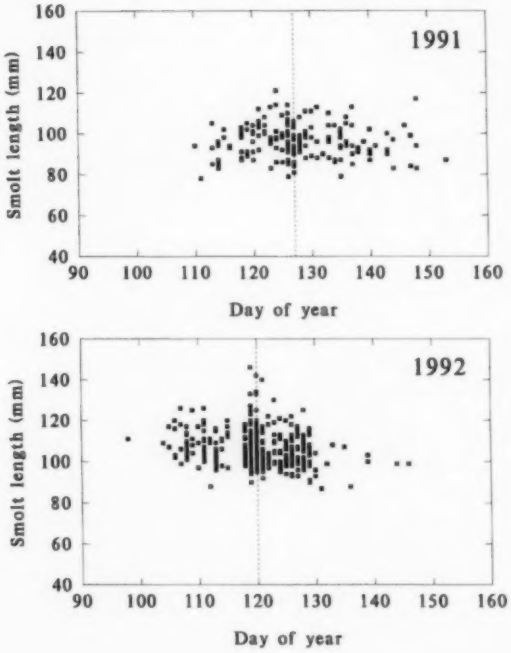


Fig. 7. Smolt length and date of migration of coho salmon tagged as underyearlings in Big Beef Creek. Broken lines indicate median migration dates. The 2-day gap in migration in 1992 resulted from retention of all fish without counting when tagging equipment malfunctioned.



Hunt (1969) attributed the positive relationship observed between brook trout (*Salvelinus fontinalis*) size and survival to energetic efficiency at low temperatures rather than size-selective predation. Experiments in semicontrolled settings with rainbow trout (*O. mykiss*) also related the higher survival of larger fish to relatively warm temperatures and the presence of rocky cover (which tended to elevate temperatures; Smith and Griffith 1994). We do not know the causes of mortality in Big Beef Creek, but the mild temperature regime (rarely below 4°C: Williams 1970) makes it unlikely that thermal stress was a major factor. Avian predation was observed in an experimental channel adjacent to the creek in the summer (Spalding et al. 1995), and the river otters present likely prey on salmonids as well (Dolloff 1993). Size-selective predation by birds, mammals, or fishes (e.g., sculpins, *Cottus* spp., or cutthroat trout, *Oncorhynchus clarki*) may have been responsible for the higher survival rate of larger salmon.

The size advantage of juvenile coho at the end of the summer may have ramifications for their entire lives. It initially affected their likelihood of over-winter survival and size as smolts. Marine survival is positively correlated with size within year-classes of coho salmon (Mathews and Ishida 1989; Holtby et al. 1990) and other salmonids such as chum salmon (*Oncorhynchus keta*; Healey 1982), sockeye salmon (Henderson and Cass 1991; Koenings et al. 1993), cutthroat trout (Tipping and Blankenship 1993), and steelhead trout (Ward and Slaney 1988; Ward et al. 1989). We did not observe a relationship between smolt length and date of capture at the weir. However, there is evidence from other years that large size is associated with early emigration from Big Beef Creek (Seiler et al. 1981, 1984), consistent with other studies of coho salmon (Irvine and Ward 1989) and anadromous brown trout (Bohlin et al. 1993). Marine survival is also strongly related to the time of seawater entry (e.g., coho, Bilton et al. 1982; Thedinga and Koski 1984; Morley et al. 1988), hence indirectly to smolt size.

Several factors may have affected the difference in survival between years. In 1991 we tagged fish above the lake, and they experienced particularly high survival rates. Even without these fish, however, the survival rate of fish from reaches below the lake was higher in 1991. The fish tagged were larger in 1991 than 1990, which might have improved their survival rate. However, for a given size-class, survival differed between years (Fig. 2), indicating that factors other than habitat conditions and fish size affected mortality. Environmental conditions varied between the years. Most notably, the maximum daily flow during the winter rearing period of the 1990 group was almost twice that experienced by the 1991 group (Lestelle et al. 1993). High flow could have resulted in either mortality within the river or displacement from it. In contrast to coastal systems such as Big Beef Creek, low winter flows have been indirectly linked to poor survival of Atlantic salmon in rivers experiencing very cold winters and low flow rates (Gibson and Myers 1988; Hvidsen 1993). We do not know which year's survival rate (if either) was typical of this stream, and the specific causes of mortality are not known. However, the survival rates (25 and 46%) were comparable with values from the literature reviewed by Murphy et al. (1984): Southeast Alaska, 26 and 35%; British Columbia, 35%; Washington, 31 and 80%; Oregon, 40, 51, 54, and 86%.

The second major finding was the relationship between habitat complexity (abundance of LWD and number of discrete habitat units per length of stream reach or pool spacing) at the end of the summer and over-winter survival. The affinity of coho salmon for woody debris increases in winter (Bustard and Narver 1975; Heifetz et al. 1986; Murphy et al. 1986;

© 1996 NRC Canada

Taylor 1988), but we did not assume that the salmon spent the winter in the habitat units where they were captured and released in October. Indeed, the strongest correlations with survival were not with the complexity of the fish's immediate environment (i.e., habitat unit) but rather with the 500 m below the capture point. Movement during winter is known in other salmonids (e.g., Atlantic salmon: Cunjak and Randall 1993) and coho salmon tend to move downstream in fall to overwinter in side channels, ponds, and sloughs (Carnation Creek: Brown and Hartman 1988; interior British Columbia: Swales et al. 1986; coastal Washington: Peterson 1982; coastal Oregon: Nickelson et al. 1992), though upstream movement from stream–estuary ecotones has also been documented (Murphy et al. 1984). Off-channel and side-channel habitat is limited in Big Beef Creek, and we assumed that the coho salmon survived the winter and smolted from main-channel habitat. The superior survival and growth of coho salmon marked above the lake is consistent with studies (e.g., Cederholm et al. 1988; Cederholm and Scarlett 1991) documenting excellent coho salmon over-winter growth and survival in small constructed ponds. However, the wetlands above the lake may also have provided suitable rearing locations. We assumed that the fish captured and released below the lake would not ascend the fishway into the lake because the fishway is designed to pass adults, not juveniles. However, 13 fish from the habitat unit closest to the lake displayed the high growth rate of "lake" fish, and we consider it possible that they moved upstream and overwintered in the lake or its associated wetlands.

One important factor that confounded our interpretation of the role of habitat complexity in over-winter survival was the tendency for simpler reaches (i.e., those with lower volumes of large wood and fewer habitat units per length of stream below them) to be situated near the mouth of the stream. Channelization in 1969 simplified the habitat in the lowermost 800 m of Big Beef Creek. Even though the stream soon began to reconfigure itself into the former channel pattern (Cederholm 1972), the lack of LWD may be a persistent effect. Fish moving downstream in fall from the lower reaches may have left the stream before they were able to find suitable habitat whereas fish starting farther upstream had a better chance of finding habitat before they reached the mouth of the stream. This would imply that the correlation of survival and habitat complexity resulted only from the location of the simple habitats in the lower part of the stream. However, Tschaplinski and Hartman (1983) reported that structurally complex reaches of Carnation Creek retained more juvenile coho salmon during the winter than simpler reaches, so our reaches in the lower portion of the stream might have produced higher survival rates had they been more complex.

We used linear regression to test for relationships of habitat features with salmon survival, but the underlying patterns are unlikely to be linear. Beyond some threshold, increasing habitat complexity and distance upriver would presumably not be associated with increased survival. The strong correlations between LWD abundance and habitat complexity are consistent with the established role of LWD in structuring stream channels (Bilby and Ward 1991; Montgomery et al. 1995). However, the multiple regression results indicated that survival was controlled more by habitat complexity than the LWD per se, consistent with observations by Shirvell (1990) and McMahon and Hartman (1989) that the behavior of coho salmon was keyed to the velocity regimes created by the LWD rather than the LWD itself.

In addition, there were two habitat features at the reach level whose significant correlations with survival were almost certainly spurious, artifacts of the covariation among features within Big Beef Creek. Gradient was positively correlated with survival and the mean depth of habitat units below the study unit was negatively correlated with survival, probably because the deeper, low-gradient lower portion of the stream was structurally simple and near the mouth. All other things being equal, we would expect low-gradient channels with deep pools to favor over-winter survival. The existence of these paradoxical relationships highlights the question of which stream habitat features best reveal the health of a stream and the effects of land management. We conclude that assessment and monitoring protocols should be organized to produce information at a spatial scale appropriate to the life-history traits of the animals and the covariation of physical features unique to each stream.

Emigration in winter from a stream the size of Big Beef Creek would normally lead fry into the main stem of a large river, whereas in Big Beef Creek they would enter directly into Hood Canal. Such fry might not survive but in any case would not be captured at the fence in spring and so would have been classified as mortalities. The ambiguity between premature emigration and mortality in our study highlights the overall uncertainty about the causes of winter mortality. Some combination of size-biased predation and resistance to displacement by floods may explain the superior survival of larger fish. It would require a study in a stream with a different configuration of habitat units or a detailed study of the movements of individual fish to disentangle the relationships between size, proximity to the stream's mouth, habitat complexity, and survival. However, in the absence of such information it seems prudent to assume that some aspect of habitat complexity enhanced the over-winter survival of coho salmon and that management practices should encourage continued functioning and succession of riparian forests (Beschta 1991) to maintain and enhance this complexity.

Acknowledgments

We thank Shelly Spalding, Andrew Hendry, David Brastow, Larry Dominguez, Gordy George, Phil Roni, Coleman Byrnes, Steven Neuhauser, and Clair Landry for assistance with fieldwork. C. Jeff Cederholm, Gino Lucchetti, K.V. Koski, Thomas Brown, and an anonymous reviewer provided helpful comments on the manuscript. The research was funded by grants from the Washington Department of Natural Resources, the U.S. Forest Service, the Bonneville Power Administration, and the H. Mason Keeler Endowment.

References

Achord, S., Kamikawa, D.J., Sandford, B.P., and Matthews, G.M. 1995*a*. Monitoring the migrations of wild Snake River spring/summer chinook salmon smolts. 1993 annual report to the U.S. Department of Energy. Bonneville Power Administration, Portland, Oreg.

Achord, S., Kamikawa, D.J., Sandford, B.P., and Matthews, G.M. 1995*b*. Monitoring the migrations of wild Snake River spring/summer chinook salmon smolts. 1994 annual report to the U.S. Department of Energy. Bonneville Power Administration, Portland, Oreg.

© 1996 NRC Canada

Baglinière, J.L., Maisse, G., and Nihouarn, A. 1993. Comparison of two methods of estimating Atlantic salmon (*Salmo salar*) wild smolt production. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 189–201.

Beschta, R.L. 1991. Stream habitat management for fish in the northwestern United States: the role of riparian vegetation. Am. Fish. Soc. Symp. **10**: 53–58.

Bilby, R.E., and Bisson, P.A. 1987. Emigration and production of hatchery coho salmon (*Oncorhynchus kisutch*) stocked in streams draining an old-growth and a clear-cut watershed. Can. J. Fish. Aquat. Sci. **45**: 1397–1407.

Bilby, R.E., and Bisson, P.A. 1992. Allochthonous versus autochthonous organic matter contributions to the trophic support of fish populations in clear-cut and old-growth forested streams. Can. J. Fish. Aquat. Sci. **49**: 540–551.

Bilby, R.E., and Ward, J.W. 1989. Changes in characteristics and function of woody debris with increasing size of streams in western Washington. Trans. Am. Fish. Soc. **118**: 368–378.

Bilby, R.E., and Ward, J.W. 1991. Characteristics and function of large woody debris in streams draining old-growth, clear-cut, and second-growth forests in southwestern Washington. Can. J. Fish. Aquat. Sci. **48**: 2499–2508.

Bilton, H.T., Alderdice, D.F., and Schnute, J.T. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. **39**: 426–447.

Bisson, P.A., Nielsen, J.L., Palmason, R.A., and Grove, L.E. 1982. A system of naming habitat types in small streams, with examples of habitat utilization by salmonids during low streamflow. *In* Acquisition and utilization of aquatic habitat inventory information. *Edited by* N.B. Armantrout. Western Division, American Fisheries Society, Portland, Oreg. pp. 62–73.

Bisson, P.A., Bilby, R.E., Bryant, M.D., Dolloff, C.A., Grette, G.B., House, R.A., Murphy, M.L., Koski, K.V., and Sedell, J.R. 1987. Large woody debris in forested streams in the Pacific Northwest: past, present, and future. *In* Streamside management: forestry and fishery interactions. *Edited by* E.O. Salo and T.W. Cundy. Institute of Forest Resources, University of Washington, Seattle, Wash. pp. 143–190.

Bisson, P.A., Quinn, T.P., Reeves, G.H., and Gregory, S.V. 1992. Best management practices, cumulative effects, and long-term trends in fish abundance in Pacific Northwest river systems. *In* Watershed management. *Edited by* R.J. Naiman. Springer-Verlag, New York. pp. 189–232.

Bohlin, T., Dellefors, C., and Faremo, U. 1993. Optimal time and size for smolt migration in wild sea trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. **50**: 224–232.

Brown, T.G. 1985. The role of abandoned stream channels as overwintering habitat for juvenile salmonids. M.Sc. thesis, University of British Columbia, Vancouver, B.C.

Brown, T.G., and Hartman, G.F. 1988. Contributions of seasonally flooded lands and minor tributaries to the production of coho salmon in Carnation Creek, British Columbia. Trans. Am. Fish. Soc. **117**: 546–551.

Bustard, D.R., and Narver, D.W. 1975. Aspects of the winter ecology of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **32**: 667–680.

Cederholm, C.J. 1972. The short-term physical and biological effects of stream channelization at Big Beef Creek, Kitsap County, Washington. M.Sc. thesis, University of Washington, Seattle, Wash.

Cederholm, C.J., and Scarlett, W.J. 1982. Seasonal immigration of juvenile salmonids into four small tributaries of the Clearwater River, Washington, 1977–1981. *In* Salmon and Trout Migratory Behavior Symposium, June 3–5, 1981, Seattle, Wash. *Edited by* E.L. Brannon and E.O. Salo. School of Fisheries, University of Washington, Seattle, Wash. pp. 98–110.

Cederholm, C.J., and Scarlett, W.J. 1991. The beaded channel: a low-cost technique for enhancing winter habitat of coho salmon. Am. Fish. Soc. Symp. **10**: 104–108.

Cederholm, C.J., Scarlett, W.J., and Peterson, N.P. 1988. Low-cost enhancement technique for winter habitat of juvenile coho salmon. North Am. J. Fish. Manage. **8**: 438–441.

Chapman, D.W. 1962. Aggressive behavior in juvenile coho salmon as a cause of emigration. J. Fish. Res. Board Can. **19**: 1047–1080.

Chapman, D.W. 1966. Food and space as regulators of salmonid populations in streams. Am. Nat. **100**: 345–357.

Crawford, D.L., and Cross, B.A. 1992. Bristol Bay sockeye salmon smolt studies for 1991. Technical fisheries report No. 92–20. Alaska Department of Fish and Game, Juneau, Alaska.

Cunjak, R.A., and Randall, R.G. 1993. In-stream movements of young Atlantic salmon (*Salmo salar*) during winter and early spring. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 43–51.

Dolloff, C.A. 1986. Effects of stream cleaning on juvenile coho salmon and Dolly Varden in southeast Alaska. Trans. Am. Fish. Soc. **115**: 743–755.

Dolloff, C.A. 1993. Predation by river otters (*Lutra canadensis*) on juvenile coho salmon (*Oncorhynchus kisutch*) and Dolly Varden (*Salvelinus malma*) in southeast Alaska. Can. J. Fish. Aquat. Sci. **50**: 312–315.

Elliott, J.M. 1993. A 25-year study of production of juvenile sea-trout, *Salmo trutta*, in an English Lake District stream. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. 109–122.

Elliott, S.T. 1986. Reduction of a Dolly Varden population and macrobenthos after removal of logging debris. Trans. Am. Fish. Soc. **115**: 392–400.

Fausch, K.D., and Northcote, T.G. 1992. Large woody debris and salmonid habitat in a small coastal British Columbia stream. Can. J. Fish. Aquat. Sci. **49**: 682–693.

Gibson, R.J., and Myers, R.A. 1988. Influence of seasonal river discharge on survival of juvenile Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. **45**: 344–348.

Hartman, G., Scrivener, J.C., Holtby, L.B, and Powell, L. 1987. Some effects of different streamside treatments on physical conditions and fish population processes in Carnation Creek, a coastal rain forest stream in British Columbia. *In* Streamside management: forestry and fishery interactions. *Edited by* E.O. Salo and T.W. Cundy. Institute of Forest Resources, University of Washington, Seattle, Wash. pp. 330–372.

Healey, M.C. 1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. **39**: 952–957.

Heifetz, J., Murphy, M.L., and Koski, K.V. 1986. Effects of logging on winter habitat of juvenile salmonids in Alaskan streams. North Am. J. Fish. Manage. **6**: 52–58.

Henderson, M.A., and Cass, A.J. 1991. Effect of smolt size on smolt-to-adult survival for Chilko Lake sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **48**: 988–994.

Holtby, L.B. 1988. Effects of logging on stream temperatures in Carnation Creek, British Columbia, and associated impacts on the coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **45**: 502–515.

Holtby, L.B., Andersen, B.C., and Kadowaki, R.K. 1990. Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **47**: 2181–2194.

Hunt, R.L. 1969. Overwinter survival of wild fingerling brook trout in Lawrence Creek, Wisconsin. J. Fish. Res. Board Can. **26**: 1473–1483.

Hvidsen, N.A. 1993. High winter discharge after regulation increases production of Atlantic salmon (*Salmo salar*) smolts in the River Orkla, Norway. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 175–177.

Irvine, J.R., and Ward, B.R. 1989. Patterns of timing and size of wild coho salmon (*Oncorhynchus kisutch*) smolts migrating from

© 1996 NRC Canada

Keogh River watershed on northern Vancouver Island. Can. J. Fish. Aquat. Sci. **46**: 1086–1094.

Jonsson, N., Jonsson, B., and Hansen, L.P. 1990. Partial segregation in timing of migration of Atlantic salmon of different ages. Anim. Behav. **40**: 313–321.

Keller, E.A., and Melhorn, W.N. 1978. Rhythmic spacing and origin of pools and riffles. Geol. Soc. Am. Bull. **89**: 723–730.

Koenings, J.P., Geiger, H.J., and Hasbrouck, J.J. 1993. Smolt-to-adult survival patterns of sockeye salmon (*Oncorhynchus nerka*): effect of smolt length and geographic latitude when entering the sea. Can. J. Fish. Aquat. Sci. **50**: 600–611.

Lestelle, L.C., Rowse, M.L., and Weller, C. 1993. Evaluation of natural stock improvement measures for Hood Canal coho salmon. Technical report No. 93–1. Point No Point Treaty Council, Kingston, Wash.

Lisle, T.E. 1987. Using "residual depths" to monitor pool depths independently of discharge. U.S. For. Serv. Res. Note No. PSW–394.

Madej, M.A. 1975. Response of a stream channel to an increase in sediment load. M.Sc. thesis, University of Washington, Seattle, Wash.

Marshall, D.E., and Britton, E.W. 1990. Carrying capacity of coho salmon streams. Can. Manuscr. Rep. Fish. Aquat. Sci. No. 2058.

Mathews, S.B., and Ishida, Y. 1989. Survival, ocean growth, and ocean distribution of differentially timed releases of hatchery coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **46**: 1216–1226.

McMahon, T.E., and Hartman, G.F. 1989. Influence of cover complexity and current velocity on winter habitat use by juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **46**: 1551–1557.

Montgomery, D.R., Buffington, J.M., Smith, R.D., Schmidt, K.M., and Pess, G. 1995. Pool spacing in forest channels. Water Resour. Res. **31**: 1097–1105.

Morley, R.B., Bilton, H.T., Coburn, A.S., Brouwer, D., Van Tine, J., and Clarke, W.C. 1988. The influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity: results from studies on three brood years at Quinsam Hatchery, B.C. Can. Tech. Rep. Fish. Aquat. Sci. No. 1620.

Murphy, M.L., Thedinga, J.F., Koski, K.V., and Grette, G.B. 1984. A stream ecosystem in an old-growth forest in Southeast Alaska. Part V. Seasonal changes in habitat utilization by juvenile salmonids. *In* Fish and Wildlife Relationships in Old Growth Forests: Proceedings of a Symposium, April 12–15, 1982, Juneau, Alaska. *Edited by* W.R. Meehan, T.R. Merrell, Jr., and T.A. Hanley. American Institute for Fishery Research Biologists. pp. 89–98.

Murphy, M.L., Heifetz, J., Johnson, S.W., Koski, K.V., and Thedinga, J.F. 1986. Effects of clear-cut logging with and without buffer strips on juvenile salmonids in Alaskan streams. Can. J. Fish. Aquat. Sci. **43**: 1521–1533.

Nickelson, T.E., Beidler, W.M., Willard, M., and Willis, M.J. 1979. Streamflow requirements of salmonids. Final report for federal aid project No. AFS–62. Oregon Department of Fish and Wildlife, Clackamas, Oreg.

Nickelson, T.E., Rogers, J.D., Johnson, S.L., and Solazzi, M.F. 1992. Seasonal changes in habitat use by juvenile coho salmon (*Oncorhynchus kisutch*) in Oregon coastal streams. Can. J. Fish. Aquat. Sci. **49**: 783–789.

Peterson, N.P. 1982. Immigration of juvenile coho salmon (*Oncorhynchus kisutch*) into riverine ponds. Can. J. Fish. Aquat. Sci. **39**: 1308–1310.

Peterson, N.P., Prentice, E.F., and Quinn, T.P. 1994. Comparison of sequential coded wire and passive integrated transponder tags for assessing overwinter growth and survival of juvenile coho salmon. North Am. J. Fish. Manage. **14**: 870–873.

Robison, E.G., and Beschta, R.L. 1990. Characteristics of coarse woody debris for several coastal streams of southeast Alaska, USA. Can. J. Fish. Aquat. Sci. **47**: 1684–1693.

Scarlett, W.S., and Cederholm, C.J. 1984. Juvenile coho salmon fall–winter utilization of two small tributaries of the Clearwater River, Jefferson County, Washington. *In* Proceedings of the Olympic Wild Fish Conference, March 23–25, 1983, Port Angeles, Wash. *Edited by* J.M. Walton and D.B. Houston. Peninsula College, Port Angeles, Wash. pp. 227–242.

Scrivener, J.C., and Andersen, B.C. 1984. Logging impacts and some mechanisms that determine the size of spring and summer populations of coho salmon fry (*Oncorhynchus kisutch*) in Carnation Creek, British Columbia. Can. J. Fish. Aquat. Sci. **41**: 1097–1105.

Seiler, D., Neuhauser, S., and Ackley, M. 1981. Upstream/downstream salmonid trapping project, 1977–1980. Progress report No. 144. Washington Department of Fisheries, Olympia, Wash.

Seiler, D., Neuhauser, S., and Ackley, M. 1984. Upstream/downstream salmonid trapping project, 1980–1982. Progress report No. 200. Washington Department of Fisheries, Olympia, Wash.

Shirvell, C.S. 1990. Role of instream rootwads as juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*O. mykiss*) cover habitat under varying streamflows. Can. J. Fish. Aquat. Sci. **47**: 852–861.

Smith, R.W., and Griffith, J.S. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. Trans. Am. Fish. Soc. **123**: 747–756.

Smoker, W.A. 1955. Effects of streamflow on silver salmon production in western Washington. Ph.D. dissertation, University of Washington, Seattle, Wash.

Spalding, S., Peterson, N.P., and Quinn, T.P. 1995. Summer distribution, survival and growth of juvenile coho salmon, *Oncorhynchus kisutch*, under varying experimental conditions of brushy instream cover. Trans. Am. Fish. Soc. **124**: 124–130.

Swales, S., Lauzier, R.B., and Levings, C.D. 1986. Winter habitat preferences of juvenile salmonids in two interior rivers in British Columbia. Can. J. Zool. **64**: 1506–1514.

Swales, S., Caron, F., Irvine, J.R., and Levings, C.D. 1988. Overwintering habitats of coho salmon (*Oncorhynchus kisutch*) and other juvenile salmonids in the Keogh River system, British Columbia. Can. J. Zool. **66**: 254–261.

Symons, P.E.K. 1979. Estimated escapement of Atlantic salmon (*Salmo salar*) for maximum smolt production in rivers of different productivity. J. Fish. Res. Board Can. **36**: 132–140.

Taylor, E.B. 1988. Water temperature and velocity as determinants of microhabitats of juvenile chinook and coho salmon in a laboratory channel. Trans. Am. Fish. Soc. **117**: 22–28.

Thedinga, J.F., and Koski, K.V. 1984. A stream ecosystem in an old-growth forest in southeast Alaska. Part VI. The production of coho salmon, *Oncorhynchus kisutch*, smolts and adults from Porcupine Creek. *In* Fish and Wildlife Relationships in Old Growth Forests: Proceedings of a Symposium, April 12–15, 1982, Juneau, Alaska. *Edited by* W.R. Meehan, T.R. Merrell, Jr., and T.A. Hanley. American Institute for Fishery Research Biologists. pp. 99–108.

Tipping, J.M., and Blankenship, H.L. 1993. Effect of condition factor at release on smolt-to-adult survival of hatchery sea-run cutthroat trout. Prog. Fish-Cult. **55**: 184–186.

Tschaplinski, P.J., and Hartman, G.F. 1983. Winter distribution of juvenile coho salmon (*Oncorhynchus kisutch*) before and after logging in Carnation Creek, British Columbia, and some implications for overwinter survival. Can. J. Fish. Aquat. Sci. **40**: 452–461.

Walters, T.R., Carmichael, R.M., and Keefe, M. 1994. Smolt migration characteristics and mainstem Snake and Columbia River detection rates of PIT-tagged Grande Ronde and Imnaha River naturally produced spring chinook salmon. Annual progress report No. 94–36. Oregon Department of Fish and Wildlife, Portland, Oreg.

© 1996 NRC Canada

Ward, B.R., and Slaney, P.A. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. Can. J. Fish. Aquat. Sci. **45**: 1110–1122.

Ward, B.R., and Slaney, P.A. 1993. Egg-to-smolt survival and fry-to-smolt density dependence of Keogh River steelhead trout. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 209–217.

Ward, B.R., Slaney, P.A., Facchin, A.R., and Land, R.W. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. Can. J. Fish. Aquat. Sci. **46**: 1853–1858.

Williams, K.R. 1970. Some ecological investigations of Big Beef Creek, 1966–67. M.Sc. thesis, University of Washington, Seattle, Wash.

Williams, K.R., Koski, K.V., and Salo, E.O. 1968. Big Beef Creek salmon studies. Extended abstract. *In* Research in fisheries 1967. College of Fisheries, University of Washington, Seattle, Wash. pp. 22–23.

© 1996 NRC Canada

Effects of chronic hypoxia and reduced temperature on survival and growth of burrowing mayflies, *Hexagenia limbata* (Ephemeroptera: Ephemeridae)

Andreas Winter, Jan J.H. Ciborowski, and Trefor B. Reynoldson

Abstract: *Hexagenia* nymphs are sensitive to hypoxia, and their abundance has been proposed as an ecosystem indicator for assessing the recovery from eutrophication of shallow, mesotrophic lakes. Acute oxygen tolerance limits are known for *Hexagenia* spp., but effects of prolonged exposure to sublethal levels of hypoxia are not. A series of 21-day laboratory experiments was conducted to determine the influence of hypoxic stress (range 2–12.6 mg/L dissolved oxygen) and temperature (range 4–20°C) on survival and growth of *Hexagenia limbata* nymphs. Oxygen and temperature together explained 89% of the variability in survival among *H. limbata*, and 71% of the variability in average growth. Survival increased with increasing oxygen concentration, reaching an asymptote at 7–8 mg/L. Survival increased with temperature to an asymptote at approximately 9.5°C. Higher temperatures magnified the effects of hypoxia on survival. Growth increased with both oxygen and temperature, and did not reach an asymptote at levels up to 12.6 mg/L oxygen and 20°C. Persistent, sublethal oxygen stress (concentrations <7 mg/L) and reduced temperature (<20°C) can influence both survival and size of *H. limbata*.

Résumé : Les nymphes d'*Hexagenia* sont sensibles à l'hypoxie, et on a proposé d'utiliser leur abondance comme indicateur écosystémique pour évaluer le rétablissement après eutrophisation des lacs mésotrophes peu profonds. On connaît les limites de la tolérance à une concentration aiguë d'oxygène chez *Hexagenia*, mais pas les effets d'une exposition prolongée à des niveaux sublétaux d'hypoxie. Nous avons mené une série d'expériences de 21 jours en laboratoire pour déterminer l'influence du stress hypoxique (fourchette 2–12,6 mg/L d'oxygène dissous) et de la température (fourchette 4–20°C) sur la survie et la croissance des nymphes d'*Hexagenia limbata*. Combinés, l'oxygène et la température expliquaient 89% de la variabilité de la survie parmi les *H. limbata* et 71% de la variabilité de la croissance moyenne. La survie augmentait avec la concentration d'oxygène, pour atteindre une asymptote à 7–8 mg/L. La survie augmentait en fonction de la température pour atteindre une asymptote à environ 9,5°C. La hausse des températures amplifiait les effets de l'hypoxie sur la survie. La croissance augmentait en fonction à la fois de l'oxygène et de la température, et n'avait pas atteint une asymptote à des niveaux de 12,6 mg/L d'oxygène et 20°C. Un stress persistant et sublétal dû à la concentration de l'oxygène (<7 mg/L) et une réduction de la température (<20°C) peuvent avoir un effet sur la survie et sur la taille d'*H. limbata*.
[Traduit par la Rédaction]

Introduction

Hexagenia mayflies were formerly abundant benthic macroinvertebrates in many shallow, mesotrophic water bodies, including extensive regions of the Great Lakes. Organic pollution eradicated *Hexagenia* spp. in western Lake Erie (Carr and Hiltunen 1965) and other basins (Schuytema and Powers 1966; Schneider et al. 1969; Howmiller and Beeton 1971) but the Lake Erie population is presently showing signs of recovery (J.J.H. Ciborowski, personal observation; L.D. Corkum, University of Windsor, personal communication; Krieger et al. 1996).

For those Great Lakes areas in which mayflies were once abundant, Reynoldson et al. (1989) have recommended the recovery of *Hexagenia* populations as an indicator of the ecosystem's rehabilitation from eutrophication. Nymphs are acutely sensitive to anoxia in that they generally die when subjected to dissolved oxygen concentrations less than ~1 mg/L for more than 24 h (Hunt 1953; Eriksen 1963). In western Lake Erie, the absence of *Hexagenia* spp. since the 1960s has been attributed to low dissolved oxygen levels (Britt 1955*a*; Reynoldson et al. 1989). This population crash occurred when unusually warm, calm conditions caused exceptionally stable thermal stratification of the water column. This prevented near-bottom waters from becoming reoxygenated, and resulted in *Hexagenia* mortality rates of up to 100% in open waters of the western basin (Britt 1955*a*). In addition to such episodes of sudden extinction, oxygen depletion will potentially have long-term effects on a *Hexagenia* population. The increased efforts required by nymphs for respiration may cause reduced growth and thus smaller adult size, which would result in lower fecundity (Clifford and Boerger 1974).

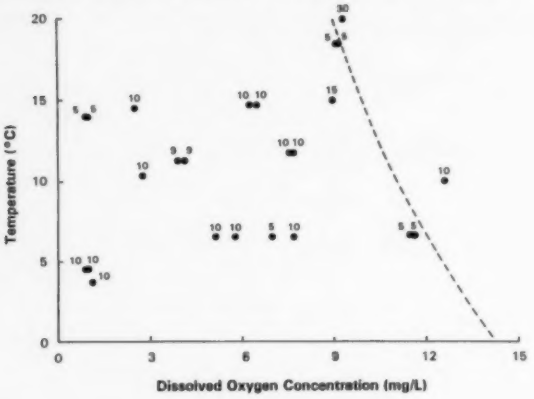
Received August 17, 1995. Accepted January 19, 1996.
J13044

A. Winter and J.J.H. Ciborowski.[1] Department of Biological Sciences and Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada.
T.B. Reynoldson. National Water Research Institute, Environment Canada, Canadian Centre for Inland Waters, Burlington, ON L7R 4A6, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: cibor@server.uwindsor.ca

© 1996 NRC Canada

Fig. 1. Plot of combinations of oxygen and temperature conditions employed. Numerals above each point indicate number of replicate rearing tubs simultaneously exposed to the oxygen–temperature combination. Broken line indicates isopleth of 100% saturation.



Western Lake Erie stratifies sporadically during summer months. Depending on their frequency and duration, these episodes can strongly influence oxygen availability in profundal zones. At the same time, temperature itself determines the solubility of oxygen (Carpenter 1966), the rate of bacterial respiration in hypolimnetic water and sediment (Blumberg and DiToro 1990), and the metabolic rates of nymphs themselves (Morgan and Wilder 1936, cited in Craven and Brown 1969).

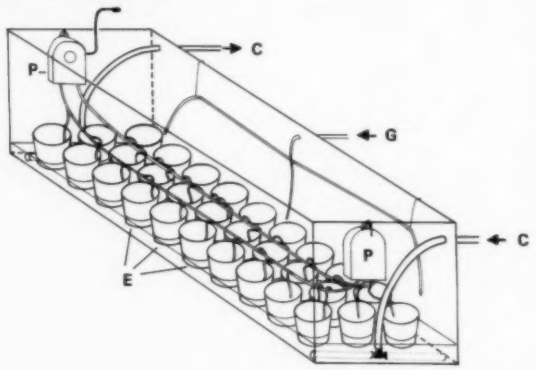
Assessing the recovery of *Hexagenia* spp. in Lake Erie and other mesotrophic lakes therefore requires an understanding of the mayflies' physiological responses to the effects of chronic, low dissolved oxygen concentrations and temperature. Here, we report results of a laboratory study evaluating the survival and growth of *Hexagenia* nymphs over a range of dissolved oxygen concentrations and temperature levels comparable with conditions that can be found in western Lake Erie. Our study provides information needed to model the recovery of mayfly communities in systems recovering from cultural eutrophication.

Materials and methods

Experimental organism

Nymphs of *Hexagenia limbata* were obtained from eggs collected from emergent females on the north shore of Lake Erie near Colchester, Ontario. The eggs were cooled and stored at 8°C following the procedures of Friesen (1981). Eggs were removed from cold storage as required, and hatched within 7 days of being returned to room temperature. Newly hatched nymphs were reared at 22–25°C in aerated aquaria containing dechlorinated tap water and Lake Erie sediment for at least 60 days. Hanes and Ciborowski (1992) found that exogenous factors did not become significant to the growth and development of *Hexagenia rigida* until they were more than 3 months old; therefore, we avoided using very young animals. Nymphs used in experiments were selected by eye for being vigorous (i.e., swimming actively in the water dish in which they were screened) and spanning a range of sizes (body length ~4–16 mm). Because both natural and laboratory-reared *Hexagenia* populations commonly exhibit great size variation (Corkum and Hanes 1992; Hanes and Ciborowski 1992), a broad size range was employed to provide a more realistic representation of nymphal population structure.

Fig. 2. Diagram of an experimental chamber containing rearing tubs (not to scale). Fewer tubs than illustrated were used in experimental trials. E, rearing tubs; P, internal atmosphere recirculating pumps; C, cooling water supply and return hoses; G, gas supply hose (from compressed nitrogen tank or line air supply). Arrows indicate direction of flow. The chamber was sealed with polyethylene sheeting.



Experimental design

Experiments consisted of 21-day trials in which nymphs were cultured at 1 of 24 combinations of fixed levels of oxygen and temperature (Fig. 1). Oxygen and temperature levels were selected to encompass the prevailing range of conditions characteristic of bottom waters of Lake Erie (Schertzer et al. 1987; Lam and Schertzer 1987; Schertzer and Sawchuk 1990). Each trial comprised a minimum of five replicate containers, which were cultured simultaneously in a common experimental chamber. Each replicate container held eight nymphs (equivalent to 1143 individuals/m^2 of substrate). This density is typical of historical values for western Lake Erie (Reynoldson et al. 1989), and nymphs grow and survive well in containers of this size (Hanes and Ciborowski 1992).

Experimental chambers and conditions

We conducted experiments in three black, 125 × 40 × 40 cm Plexiglas water baths (Corkum and Hanes 1992; hereafter called chambers), covered in transparent, utility weight (1-mil = 0.025 mm) polyethylene sheeting, sealed with masking tape to isolate the atmosphere inside (Fig. 2). Within these chambers, nymphs were placed in each of 10 or more 1-L, heavy polyethylene containers (bottom area 70 cm^2 × 15 cm tall; hereafter called tubs), containing a 4 cm depth of Lake Erie sediment and 11 cm depth of dechlorinated City of Windsor water. Sediment had been collected by box corer from two locations in western Lake Erie (stations 357 and 358 of Reynoldson and Hamilton 1993). Sediments were stored at 4°C for several months until required and then autoclaved. Sediments were predominantly silt (60–70% of particles <40 μm, determined by hydrometry; loss on ignition 6–7% (Winter 1994)).

Water temperature was controlled by circulating water through the chambers, each cooled (when appropriate) by Aquachiller water refrigeration units (Jewel Industries Inc., Chicago, Ill). Temperature conditions were also stabilized prior to the start of each trial.

Rearing tubs were individually aerated by a system of capillary tubing and hypodermic needles (Corkum and Hanes 1989) fed from one of two manifolds attached to electrically powered, twin-output pumps (Model Elite 802, Rolf C. Hagen Inc., Montréal, Que.). The pumps were mounted inside the chambers on opposite walls, one attached to each end of both manifolds, thus constantly recirculating the atmosphere inside the chamber.

The atmospheric oxygen concentration inside the experimental

© 1996 NRC Canada

chambers was regulated by venting in nitrogen gas, pressurized atmospheric air, or both, through Nalgene plastic tubes supplied from either a gas cylinder (nitrogen) or the laboratory line air system (atmospheric air). In some trials, air and nitrogen were separately injected directly into opposite manifolds connecting the two electric air pumps. Although the gases subsequently mixed within the chamber, this separate delivery system ensured that one row of five or more rearing tubs, fed from the nitrogen-injected manifold, would always receive a higher incipient level of nitrogen, and the other row of five or more tubs would receive a higher level of air. Depending on the incoming pressure, this arrangement sustained differences of as much as 0.7 mg/L O_2 between separate rows of rearing tubs within the same chamber, for the 21-day duration of an experiment. These two oxygen treatments, each consisting of a row of five or more tubs, were then analysed as separate trials. Thus, a single chamber generated either one or two of the 24 oxygen–temperature combinations (Fig. 1), depending on whether gas was fed to the chamber (as illustrated in Fig. 2; one trial) or directly to the manifolds (two trials). Oxygen concentrations were stabilized for at least 12 h prior to the start of every trial by adjusting the flow of nitrogen and air.

One millilitre of a mixture of baker's yeast (15 g), Tetramin fish food flakes (20 g), alfalfa powder (15 g), and water (500 mL) (Hanes and Ciborowski 1992) was added to each rearing tub prior to the start of the trial. The food mixture was pipetted just beneath the water surface, allowing it to settle evenly over the sediment. Nymphs were not fed again during a trial, to minimize exposure to outside air.

Experimental procedure

The nymphs destined for each rearing tub were individually marked by clipping a combination of caudal filaments (Ciborowski et al. 1992). With three caudal filaments per nymph, $2^3 = 8$ clipping patterns are available (Ciborowski et al. 1992). The particular clipping pattern was randomly assigned to each of the eight individuals allocated to a rearing tub. Such clipping does not influence growth or survival of the nymphs (J.J.H. Ciborowski, E.C. Hanes, A. Winter, and L.D. Corkum, unpublished data; Winter 1994). Marked nymphs were then photographed against a 1-cm^2 grid. Head width (to the outside of the eyes) of each individual was determined by measuring the size of its photographic image to the nearest 0.04 mm at 25× magnification with an ocular micrometer in a dissecting microscope (Hanes 1992).

Immediately before the nymphs were introduced, the oxygen concentration in each rearing tub was measured using either a YSI model 57 oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio) or the Winkler titration method (Wetzel and Likens 1991). Oxygen concentrations were measured to the nearest 0.2 mg/L. Temperature was also measured in each rearing tub, at the sediment–water interface, using thermometers graduated to 0.1°C. The chamber was then resealed and maintained at the required inflow of air–nitrogen and cooling water for the 21-day duration of the trial. At the conclusion of the trial, oxygen and temperature measurements were repeated in each rearing tub as soon as the chamber was opened. All remaining live nymphs were then retrieved and photographed, and images of their head width were remeasured for determination of growth (see below).

Determination of growth and survival

Survival was expressed as the proportion of the eight nymphs per rearing tub recovered alive at the end of each 21-day trial. Survival data from the five or more replicate rearing tubs within the chamber were then averaged for a given trial. Growth was calculated as the percentage change in head width ($100 \times \ln$(final head width/initial head width)) of a nymph during trial. Negative growth (value less than zero) occurred when the final head width of a nymph was less than the initial head width. The values for all surviving nymphs in a rearing tub were averaged. Growth in a trial was expressed as the mean of the means of each rearing tub in which at least one nymph survived.

Table 1. Nonlinear and multiple regression models predicting survival and growth of *Hexagenia limbata*.

Equation no.	Constant	$[O_2]$	Temp.^{2}	$[O_2]\times$ Temp.	IHW	R^2
			Survival			
1	0.825	−0.482	−0.043	—	—	0.89
2	0.755	−0.212	—	—	—	0.84
3	0.700	—	−0.039	—	—	0.38
			Growth			
4	−1.739	—	0.012	0.036	—	0.71
5	6.581	—	0.008	0.048	−4.351	0.77
6	−0.446	0.501	—	—	—	0.25
7	−1.035	—	0.025	—	—	0.57

Note: Nonlinear models (survival) are in the form $y = a \cdot (1 - e^{b \cdot X1}) \cdot (1 - e^{c \cdot X2})$. Polynomial models (growth) are expressed as $y = a + b \cdot X1 + c \cdot X2 + \cdots$.

Survival and growth data were each analysed by forward stepwise multiple regression analysis (Draper and Smith 1981). Independent variables included in the analyses were oxygen concentration (mg/L), temperature (°C), (oxygen concentration)^2, (temperature)^2, and oxygen × temperature. Quadratic terms were included to detect possible nonlinearity of the relationships, which would have indicated maximal growth or survival at some intermediate level of one or more of the independent variables.

For survival, stepwise multiple regression was initially used to select significant variables, but the polynomial function was subsequently changed to a nonlinear regression function as this produced a saturation curve, which was biologically more realistic. For growth, we additionally tested initial head width (mm) as an independent factor in the regression analysis.

Because field surveys and records of physical data are often incomplete, oxygen and temperature measurements may not both be available for a given location or period of interest. Therefore, we also calculated regressions using oxygen and temperature variables separately.

Results

Survival

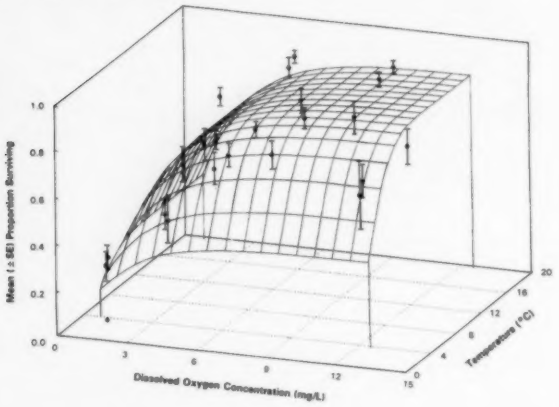
Survival increased asymptotically with increasing oxygen concentration up to the level of approximately 7–8 mg/L (eq. 1 in Table 1; Fig. 3), well above the 24-h tolerance thresholds of 0.8–1.0 mg/L reported by Hunt (1953) and Eriksen (1963). The influence of temperature on survival was positive as well, and reached an asymptote at approximately 9.5°C (Fig. 3). The mortality curve with respect to either oxygen or temperature became increasingly steep as the complementary independent variable increased in magnitude (compare opposite edges of the response surface in Fig. 3). As a single independent variable, oxygen concentration (eq. 2 in Table 1) provided a better fit by nonlinear regression than did temperature (eq. 3 in Table 1; Figs. 4*a*, 4*b*).

Growth

Results of a nested analysis of variance (ANOVA) showed variation in nymphal growth among treatments ($F_{[23,1141]} = 31.90$, $p < 0.0001$) but not among replicate tubs within treatments ($F_{[185,1141]} = 1.18$, $p > 0.05$). Therefore, a regression model for growth as a function of oxygen and temperature was calculated with all replicate tubs per treatment pooled (eq. 4 in Table 1; Fig. 5).

© 1996 NRC Canada

Fig. 3. Response surface plot of eq. 1 (Table 1) showing influence of oxygen concentration and temperature on survival of *Hexagenia* nymphs. Actual experimental data (means of replicate rearing tubs ± 1 SE) are superimposed.



The interaction of oxygen × temperature removed approximately 44% of the total variation in growth among treatments, and (temperature)^2 accounted for a further 27%. The inclusion of initial head width in the regression analysis produced an additional significant increase in the coefficient of determination (eq. 5 in Table 1). However, because growth was defined as the log ratio of a nymph's final head width to its initial head width, this improvement came mostly as a result of self-correlation. Growth, in fact, was a significant function of initial size alone (Winter 1994). The inclusion of initial head width in the model thus contributes little explanatory power to the growth response of *Hexagenia* nymphs, but improves the ability to predict growth for individuals whose size can be determined beforehand.

The calculation of regressions using oxygen and temperature separately is represented in eqs. 6 and 7 in Table 1 (Figs. 6*a*, 6*b*). Temperature was the most important predictor variable for growth, but the influences of both temperature and oxygen were statistically significant.

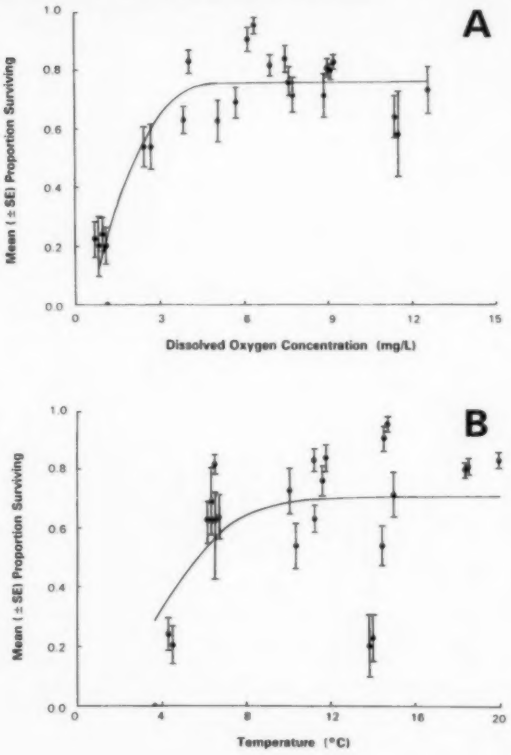
Discussion

Survival

Survival of *Hexagenia* nymphs over 21 days was positively related to the concentration of dissolved oxygen, up to an asymptote at the level of approximately 8 mg/L. This result implies that even light to moderate oxygen stress can increase the mortality rate of the nymphs, if the hypoxic conditions persist.

It has not been previously reported that prolonged sublethal hypoxia influences survival rates among *Hexagenia* spp. Experimental investigations with aquatic invertebrates have usually been restricted to evaluating acute tolerance limits. However, the history of the mayfly population in western Lake Erie suggests that the response relationship to moderate oxygen stress may be as important as the tolerance threshold. The numbers of *Hexagenia* spp. in the western basin of Lake Erie fluctuated considerably between 1928 and 1960 (Fig. 1 of Reynoldson et al. 1989), dropping almost to zero in 1953. However, young nymphs were reported in 1954 (Britt 1955*b*),

Fig. 4. Mean (±1 SE) survival of *Hexagenia* nymphs as a function of (*a*) oxygen concentration and (*b*) temperature.



and this recovery suggests that a single, catastrophic event is not enough to completely eradicate a population (perhaps because eggs are resistant to anoxia (Hunt 1953)).

Our data are consistent with the events of 1953–1954 (Britt 1955*a*, 1955*b*). Even the harshest hypoxic conditions in our experiments (0.82–0.84 mg O_2/L) killed fewer than 80% of nymphs over 3 weeks. Given the ephemeral and intermittent nature of thermal stratification in large, shallow waters like western Lake Erie (Bartish 1984), it is unlikely that a single episode of total anoxia could cover the whole western basin for long enough to have eradicated the entire mayfly population there at one time. High levels of mortality are thus more likely due to prolonged exposure to oxygen stress.

In our study, dead nymphs were recovered after 21 days in various states of decomposition (or not at all), indicating that individuals died over a range of time intervals. Oxygen stress thus increased the probability of mortality rather than inflicting outright mass mortality. This implies that the duration as well as the severity of an episode of hypoxia can affect the survival of *Hexagenia* populations. Natural recovery of populations may therefore be anticipated even after brief periods of anoxia.

Nymphs that were initially larger were significantly more likely to survive than small nymphs (as determined by analyses of maximum-likelihood estimates for logistic regressions of survivorship versus initial body length for larvae in individual rearing tubs (Winter 1994)).

Mean survival increased with increasing temperature as

© 1996 NRC Canada

Fig. 5. Response surface plot of eq. 4 (Table 1) showing the influence of oxygen concentration and temperature on growth of *Hexagenia* nymphs. Actual experimental data (means of replicate rearing tubs ± 1 SE) are superimposed.

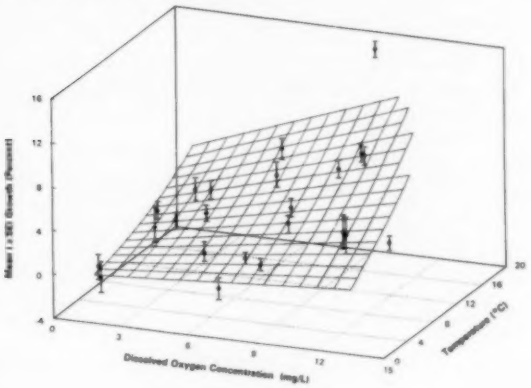
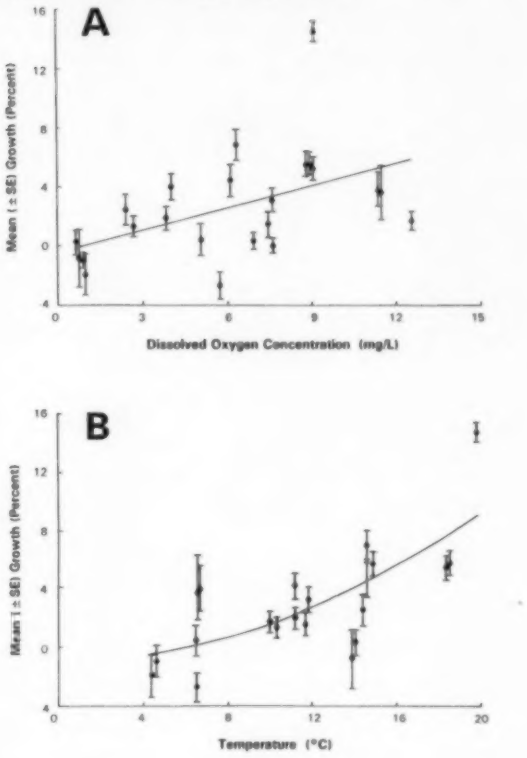


Fig. 6. Mean (±1 SE) growth of *Hexagenia* nymphs as a function of (*a*) oxygen concentration and (*b*) temperature.



well, up to an asymptote at approximately 9.5°C. This is close to the temperature generally regarded as the developmental threshold for *Hexagenia* spp. (10°C; Heise et al. 1987 and references therein; Giberson and Rosenberg 1992). As temperatures decreased below this point, the increasing likelihood of developmental arrest thus correlates with increasing chances of mortality. Nevertheless, temperature by itself explained only 38% of the variability in survival among treatments, as opposed to 84% for oxygen. This suggests that cold stress also increases the probability of mortality indirectly, rather than directly. Developmental stasis perhaps inhibits recovery from tissue damage or other injury, leaving nymphs more vulnerable to death by other causes.

In our study, high temperatures produced a steeper mortality response to oxygen concentration. A reduction in dissolved oxygen concentration at a high temperature would thus be more harmful to nymphs than the same degree of hypoxia at a lower temperature. In a water body such as western Lake Erie, this has important implications for the survival of a mayfly population. Stratification leading to hypoxia in these waters occurs in the summertime, when temperature is high (Bartish 1984). A temperature a few degrees celcius higher or lower during hypoxia might make the difference between complete mortality or sustainable survival. Notably, the severe basin-wide extinction in 1953 followed stratification estimated at less than 14 days duration, but at the elevated bottom temperature of 24°C (Britt 1955*a*).

Our results suggest that a summer regime of ≥8 mg/L oxygen and ≥10°C is adequate to sustain *Hexagenia* nymphs without limiting their survival. Persistent, sublethal oxygen stress (concentrations <7 mg/L) and reduced temperature (<20°C) measurably reduce *Hexagenia* survival. We believe we can now begin to predict the recovery and survival potential of mayfly populations, given daily estimates of temperature and oxygen at the level of the substrate.

Growth

The interaction of oxygen and temperature was the single most important determinant of nymphal growth over 21 days: as temperature rose, metabolic rates increased, resulting in faster growth, presumably creating higher oxygen demand for *Hexagenia* nymphs. Oxygen and temperature also influenced growth singly, but their predictive power increased when they were considered together. Unlike survival, growth did not approach an asymptote with respect to either oxygen or temperature, over the range of conditions used. For temperature, this result was expected. Previous work has shown that the maximum growth temperatures for *Hexagenia* spp. are just above 20°C (Wright and Mattice 1981; Wright et al. 1982; Corkum and Hanes 1992); temperatures in our study thus represent a range below the optimal zone.

The observed relationship between growth and oxygen concentration has not been previously reported. Average survival peaked at around 7–8 mg/L dissolved oxygen. It is noteworthy that the capacity for growth continued to increase above this point. In warm, shallow water bodies such as western Lake Erie, the water column mixes easily (Bartish 1984) and a storm or high winds can quickly entrain oxygen-rich surface waters to the sediment interface. Our results suggest that *Hexagenia* nymphs can exploit such oxygen conditions to produce faster growth than would occur under calm conditions.

The mechanism that stimulated nymphal growth at high, saturated oxygen levels in our experiments probably involves a reduction in the need to actively ventilate (Eriksen 1963), or an increase in the efficiency of feeding. The energy requirements of processing and assimilating food demand oxygen, and positive correlations between oxygen availability and food

© 1996 NRC Canada

consumption rates have been determined in various fish species (Warren 1971; Muir and Niimi 1972; Beamish 1974; Ross et al. 1992). The same principle may be valid in mayflies.

Many of the animals reared under low oxygen or temperature conditions were smaller when recovered after 21 days than when introduced into their rearing tubs. Most of the animals displaying negative growth were dead, but a number of living nymphs became smaller as well, indicating that use of stored reserves prior to moulting to the next instar is one end of the continuum of growth responses, and not a qualitative symptom specifically associated with moribund or severely stressed animals.

It would be instructive to determine, in future experiments, the full range over which the positive relationship between growth, oxygen, and temperature extends. In our experiments, the predictive power of oxygen and temperature was substantially lower for growth than for survival.

Acknowledgements

We thank L.D. Corkum for the loan of water-bath chambers. B. Manny provided many constructive comments on the manuscript. This research was funded from grants from Environment Canada to T.B.R. and from the Natural Sciences and Engineering Research Council of Canada to J.J.H.C.

References

Bartish, T.M. 1984. Thermal stratification in the western basin of Lake Erie: its characteristics, mechanisms of formation, and chemical and biological consequences. Technical report No. 289. Centre for Lake Erie Area Research, Columbus, Ohio.

Beamish, F.W.H. 1974. Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. J. Fish. Res. Board Can. **31**: 1763–1769.

Blumberg, A.F., and DiToro, D.M. 1990. Effects of climate warming on dissolved oxygen concentrations in Lake Erie. Trans. Am. Fish. Soc. **119**: 210–223.

Britt, N.W. 1955*a*. Stratification in western Lake Erie in summer of 1953: effects on the *Hexagenia* (Ephemeroptera) population. Ecology, **36**: 239–244.

Britt, N.W. 1955*b*. *Hexagenia* (Ephemeroptera) population recovery in western Lake Erie following the 1953 catastrophe. Ecology, **36**: 520–522.

Carpenter, J.H. 1966. New measurements of oxygen solubility in pure and natural water. Limnol. Oceanogr. **11**: 264–277.

Carr, J.F., and Hiltunen, J.K. 1965. Changes in the bottom fauna of western Lake Erie from 1930 to 1961. Limnol. Oceanogr. **10**: 551–569.

Ciborowski, J.J.H., Hanes, E.C., and Corkum, L.D. 1992. Standardized rearing materials and procedures for *Hexagenia*, a benthic aquatic bioassay organism. Semiannual report prepared for the Research Advisory Committee, Ontario Ministry of the Environment, Toronto, Ont.

Clifford, H.F., and Boerger, H. 1974. Fecundity of mayflies (Ephemeroptera), with special reference to mayflies of a brown-water stream of Alberta, Canada. Can. Entomol. **106**: 1111–1119.

Corkum, L.D., and Hanes, E.C. 1989. A laboratory aeration system for rearing aquatic invertebrates. Entomol. News, **100**: 169–172.

Corkum, L.D., and Hanes, E.C. 1992. Effects of temperature and photoperiod on larval size and survivorship of a burrowing mayfly (Ephemeroptera, Ephemeridae). Can. J. Zool. **70**: 256–263.

Craven, R.E., and Brown, B.E. 1969. Ecology of *Hexagenia* naiads (Insecta, Ephemeridae) in an Oklahoma reservoir. Am. Midl. Nat. **82**: 346–358.

Draper, N.R., and Smith, H. 1981. Applied regression analysis. 2nd ed. John Wiley & Sons, New York.

Eriksen, C.H. 1963. Respiratory regulation in *Ephemera simulans* Walker and *Hexagenia limbata* (Serville) (Ephemeroptera). J. Exp. Biol. **40**: 455–467.

Friesen, M.K. 1981. *Hexagenia rigida* (McDunnough). *In* Manual for the culture of selected freshwater invertebrates. *Edited by* S.G. Lawrence. Can. Spec. Publ. Fish. Aquat. Sci. No. 54. pp. 127–142.

Giberson, D.J., and Rosenberg, D.M. 1992. Effects of temperature, food quantity, and nymphal rearing density on life-history traits of *Hexagenia* (Ephemeroptera: Ephemeridae) from Southern Indian Lake, Manitoba, Canada. J. North Am. Benthol. Soc. **11**: 181–193.

Hanes, E.C. 1992. Life history characteristics and size variation of the burrowing mayfly *Hexagenia* (Ephemeroptera: Ephemeridae): maternal vs. environmental constraints. M.Sc. thesis, University of Windsor, Windsor, Ont.

Hanes, E.C., and Ciborowski, J.J.H. 1992. Effects of density and food limitation on size variation and mortality of larval *Hexagenia rigida* (Ephemeroptera: Ephemeridae). Can. J. Zool. **70**: 1824–1832.

Heise, B.A., Flannagan, J.F., and Galloway, T.D. 1987. Life histories of *Hexagenia limbata* and *Ephemera simulans* (Ephemeroptera) in Dauphin Lake, Manitoba. J. North Am. Benthol. Soc. **6**: 230–240.

Howmiller, R.P., and Beeton, A.M. 1971. Biological evaluation of environmental quality, Green Bay, Lake Michigan. J. Water Pollut. Control Fed. **43**: 123–133.

Hunt, B.P. 1953. The life history and economic importance of a burrowing mayfly, *Hexagenia limbata*, in southern Michigan lakes. Bulletin of the Institute of Fisheries Research No. 4. Michigan Department of Conservation, Lansing, Mich.

Krieger, K.A., Schloesser, D., Manny, B., Trisler, C., Heady, S., Ciborowski, J.J.H., and Muth, K. 1996. Evidence of the recovery of burrowing mayflies (Ephemeroptera: Ephemeridae: *Hexagenia*) in western Lake Erie. J. Great Lakes Res. **22**: 254–263.

Lam, D.C.L., and Schertzer, W.M. 1987. Lake Erie thermocline model results: comparison with 1967–1982 data and relation to anoxic occurrences. J. Great Lakes Res. **13**: 757–769.

Morgan, A.H., and Wilder, J.F. 1936. The oxygen consumption of *Hexagenia recurvata* during the winter and early spring. Physiol. Zool. **9**: 153–169.

Muir, B.S., and Niimi, A.J. 1972. Oxygen consumption of the euryhaline fish aholehole (*Kuhlia sandvicensis*) with reference to salinity, swimming and food consumption. J. Fish. Res. Board Can. **29**: 67–77.

Reynoldson, T.B., and Hamilton, A.L. 1993. Historic changes in populations of burrowing mayflies (*Hexagenia limbata*) from Lake Erie based on sediment tusk profiles. J. Great Lakes Res. **19**: 250–257.

Reynoldson, T.B., Schloesser, D.W., and Manny, B.A. 1989. Development of a benthic invertebrate objective for mesotrophic Great Lakes waters. J. Great Lakes Res. **15**: 669–686.

Ross, L.G., McKinney, R.W., Cardwell, S.K., Fullarton, J.G., Roberts, S.E.J., and Ross, B. 1992. The effects of dietary protein content, lipid content and ration level on oxygen consumption and specific dynamic action in *Oreochromis niloticus* L. Comp. Biochem. Physiol. **103**: 573–578.

Schertzer, W.M., and Sawchuk, A.M. 1990. Thermal structure of the lower Great Lakes in a warm year: implications for the occurrence of hypolimnion anoxia. Trans. Am. Fish. Soc. **119**: 195–209.

Schertzer, W.M., Saylor, J.H., Boyce, F.M., Robertson, D.G., and Rosa, F. 1987. Seasonal thermal cycle of Lake Erie. J. Great Lakes Res. **13**: 468–486.

Schneider, J.C., Hooper, F.H., and Beeton, A.M. 1969. The distribu-

© 1996 NRC Canada

tion of benthic fauna in Saginaw Bay, Lake Huron. Proc. 12th Conf. Great Lakes Res. pp. 80–90.

Schuytema, G.S., and Powers, E. 1966. The distribution of benthic fauna in Lake Huron. Proc. 9th Conf. Great Lakes Res. pp. 155–163.

Warren, C.E. 1971. Biology and water pollution control. W.B. Saunders Company, Philadelphia, Pa.

Wetzel, R.G., and Likens, G.E. 1991. Limnological analyses. 2nd ed. Springer-Verlag, New York.

Winter, A. 1994. Influence of oxygen stress, temperature and density on the growth and survivorship of *Hexagenia* nymphs (Ephemeroptera: Ephemeridae). M.Sc. thesis, University of Windsor, Windsor, Ont.

Wright, L.L., and Mattice, J.S. 1981. Effects of temperature on adult size and emergence success of *Hexagenia bilineata* under laboratory conditions. J. Freshwater Ecol. **1**: 27–39.

Wright, L.L., Mattice, J.S., and Beauchamp, J.J. 1982. Effect of temperature and sex on growth patterns in nymphs of the mayfly *Hexagenia bilineata* in the laboratory. Freshwater Biol. **12**: 535–545.

© 1996 NRC Canada

Estimating fish mortalities and cohort sizes

J.E. Paloheimo and Y. Chen

Abstract: A cohort-based regression model (J.E. Paloheimo. 1980. Trans. Am. Fish. Soc. 109: 378–386) has been frequently used in fish stock assessment. With a time series of catch-at-age and effort as input data, this model yields estimates of catchability, natural mortality, and cohort sizes at recruitment using a multiple regression analysis. Limitations in the error structure have been cited as a potential problem with the method. The purpose of this study is to develop a suitable error structure for this model and evaluate the effect of such an error structure on parameter estimation. The proposed error structure depends on variances in input data that are not generally known. Using simulated data both with correct and deliberately wrong input variances we observed that the estimates were not particularly sensitive to the magnitudes of these variances, but depended primarily on the structure and signs of elements in the variance–covariance matrix. The amended model does result in improved parameter estimates compared with the original model.

Résumé : Un modèle de régression à base de cohortes (J.E. Paloheimo. 1980. Trans. Am. Fish. Soc. 109: 378–386) a souvent été utilisé dans l'évaluation des stocks de poissons. Alimenté par une série chronologique de données sur les captures selon l'âge et sur l'effort, ce modèle donne des estimations de la capturabilité, de la mortalité naturelle et de la taille des cohortes au recrutement à l'aide d'une analyse de régression multiple. Les limitations de la structure d'erreur semblent toutefois poser un problème dans cette méthode. L'objet de notre étude a été d'établir une structure d'erreur convenant à ce modèle et d'évaluer l'effet d'une telle structure sur l'estimation des paramètres. La structure d'erreur proposée dépend des variances dans les données d'entrée, qui ne sont généralement pas connues. En nous servant de données simulées, avec des variances d'entrée à la fois correctes et délibérément fausses, nous avons observé que les estimations n'étaient pas particulièrement sensibles à l'ampleur de ces variances, mais dépendaient avant tout de la structure et des signes des éléments dans la matrice variance–covariance. Le modèle modifié permet d'obtenir des estimations des paramètres nettement meilleures que le modèle original.
[Traduit par la Rédaction]

Introduction

Age-structured fish stock assessments call for estimation of stock parameters including mortality rates and cohort sizes (Hilborn and Walters 1992). Many methods have been developed to estimate these parameters. Age-structured methods can be divided into two groups on the basis of their sources of input data: models using catch-at-age data only, and models using catch-at-age and effort data and perhaps other auxiliary information.

In the first type of models, where only catch-at-age data are used, fishing mortalities and cohort sizes are determined from catch-at-age data alone without any knowledge of fishing effort. However, some of the parameters need to be provided or assumed before others can be estimated. In Pope's cohort analysis, these assumptions include known natural mortality and terminal fishing mortality (Pope 1972). Variants of these assumptions are retained in subsequent refinements of Pope's cohort analysis by Doubleday (1976), Fournier and Archibald (1982), Pope and Shepherd (1982), Deriso et al. (1985), and others. Because of these assumptions neither the initial cohort size nor the annual fishing mortality can be estimated reliably. A late variant of the catch-at-age analysis by Paloheimo and Chen (1993) assumes only a value of the natural mortality rate and that the average (over all years) mortality rate equals the actual mortality rate over the span of years considered. If the age groups considered are fully recruited the last assumption is valid, at least asymptotically, leaving only the natural mortality rate to be guessed.

There are methods that can be used to estimate the natural mortality rate (M) when data on fishing effort are available and when a (linear) relationship between the physical effort of fishing and the resultant fishing mortality can be assumed. The validity of a linear relationship between the effort and mortality has been questioned (Paloheimo and Dickie 1964; Hilborn and Walters 1992) and a nonlinear relationship between the effort and the fishing mortality has been demonstrated for George's Bank haddock fishery (Crecco and Overholz 1990). The relationship between fishing effort and fishing mortality probably depends on the nature of the fishery and the distribution of fish and how the two change with the abundance of fish, each fishery perhaps being a case by itself. Before an attempt is made to estimate M the estimates of the fishing mortalities obtained from a cohort type of analysis should be plotted against the measures of effort to verify the relationship between the two.

Given that the fishing mortality is proportional to the estimated fishing effort there are still many statistical problems associated with estimating M that center around the error structure of the assumed catch equation. Some of the earlier models totally ignored the error structure (e.g., Paloheimo 1980), or assumed that the error is primarily in the sampling for catches-at-age (Fournier and Archibald 1982), or treated the different data points such as catches-at-age as independent observations

Received August 25, 1994. Accepted January 19, 1996.
J12554

J.E. Paloheimo[1] and Y. Chen. Department of Zoology, University of Toronto, Toronto, ON M5S 1A1, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: jyri@zoo.utoronto.ca

© 1996 NRC Canada

Table 1. Example of coding of cohorts and catches-at-age.

	Year									
	Coding of cohorts					Catches at age				
Age	1	2	3	4	5	1	2	3	4	5
1	2	3	4	5	—	3	6	9	12	—
2	1	2	3	4	5	1	4	7	10	13
3	—	1	2	3	4	—	2	5	8	11

Note: The number of age groups is 3 ($a = 2$) and the number of years is 5 ($y = 4$). To use the table note that, for example, 2 year olds in year 4 come from the 4th cohort and represent the 10th observation.

(Deriso et al. 1985). In addition, some sophisticated models estimate mortality rates in conjunction with other parameters (e.g., recruitment) and thus, potentially, create artificial links between the parameters.

A cohort-based model developed by Paloheimo (1980) can be applied to fish stock assessment when both catch-at-age and effort data are available. This model has been described as a simplified version of Doubleday's (1976) model (Hilborn and Walters 1992) and a special case of the CAGEAN model (i.e., when there is no error between the observed and effective fishing efforts) developed by Deriso et al. (1985). The model is simple and widely accessible to anyone with a large linear regression program (Hilborn and Walters 1992). It yields estimates of the catchability coefficient (q), the natural mortality rate (M), and the size of each cohort at its first entry into the fishery. Although the correlation between parameters q and M is usually high, it is possible to separate M from q if there are sufficiently large among-year variations in fishing efforts. However, like many previous models, this one assumes a very simple error structure. While this fact has been suggested as a major source of error in parameter estimation (Deriso et al. 1985; Hilborn and Walters 1992), the probable magnitude of such errors has not been quantified to date.

In this study we propose a suitable error structure for the Paloheimo model and develop a corresponding algorithm to estimate the parameters. On the basis of simulated data, we examine the effects of the lack of a suitable error structure on parameter estimation. We also examine the estimated parameters' sensitivity to the error variances assumed.

Assumed error structure

There are two formulations of the Paloheimo model; one is linear with constant age- and year-independent catchability (q) and selectivity (i.e., all fish are fully recruited), and the other is nonlinear with variable q and selectivity. The linear model was used in this study. Thus, catchability and selectivity coefficients were assumed constant across years and age groups (Paloheimo 1980). For the nonlinear model, a suitable error structure can be developed using an approach similar to that used in this study.

The following error structures were assumed for catch and effort data:

$$(1) \qquad \ln(C_{i,j}) = \ln(O_{i,j}) + \Delta C_{i,j},\ \ln(E_{i,j}) = \ln(f_j) + \Delta f_j + \Delta f_{i,j}$$

where i indexes age, j indexes year, $C_{i,j}$ and $E_{i,j}$ are the true values of catch and effort, $O_{i,j}$ and f_j are the corresponding observed values of catch and effort, Δf_j is a random error term subject to year variation, and both $\Delta C_{i,j}$ and $\Delta f_{i,j}$ are random error terms subject to age and year variation. It is assumed that the three error terms are uncorrelated with each other, are normally distributed, and have constant variances; i.e., $\Delta f_j \sim N(0,\sigma_y^2)$, $\Delta f_{i,j} \sim N(0, \sigma_a^2)$, and $\Delta C_{i,j} \sim N(0, \sigma_c^2)$. With some minor changes in what follows we could assume σ_c^2 to be age dependent.

Let $i = 1, \ldots, a+1$ and $j = 1, \ldots, n+1$. If fish are all fully recruited and catchabilities are the same among years and ages, catch in the cohort's 1st year in the fishery can be approximated as

$$\ln(C_{i,j}) = \ln(E_{i,j}) + \ln(qN_m) - \tfrac{1}{2}\, qE_{i,j} - \tfrac{1}{2}M,$$

where an approximation $\ln((1 - e^{-Z})/Z) = -Z/2$ is used. It is certainly true in the limit ($Z \to 0$) and even when Z is as large as 0.9 that the error of this approximation is only 7.5%; i.e., for instance when $Z = 0.9$ then $\ln((1 - e^{-Z})/Z) = 0.46Z$ instead of $0.5Z$. The errors resulting from this approximation are much smaller than the errors associated with catch and effort data, and can be ignored safely.

When the age group has been in the fishery longer than 1 year we can apply the exponential survival equation written as $N_{i+1,j+1} = N_{i,j}e^{-(qE_{i,j} + M)}$ giving the general linearized version of the catch equation for a cohort:

$$(2) \qquad \ln(C_{i,j}) = \ln(E_{i,j}) + \ln(qN_m) - \sum_{l=k}^{i} q\delta(l,i)E_{l,j-i+l} - M(i - k + 1/2)$$

where $\delta(l,i) = 1$ when $l \neq i$ and $\delta(i,i) = 0.5$, parameter k defines the age of entry of the cohort to the data base and is given by $k = \max(1, i-j+1)$, and N_m is the mth cohort in the data base. Using the coding scheme given in Table 1, m can be computed as $m = j - i + a$.

Replacing $E_{i,j}$ and $C_{i,j}$ with f_j and $O_{i,j}$ and using the approximation $E_{i,j} = f_j(1 + \Delta f_j + \Delta f_{i,j})$, the above equation can be rewritten as

$$(3) \qquad \ln(O_{i,j}) = \ln(f_j) + \ln(qN_m) - \sum_{l=k}^{i} q\delta(l,i)f_{j-i+l} - M(i - k + 1/2) + \mathrm{e}_{i,j}$$

where the error term $\mathrm{e}_{i,j}$ can be written as

$$(4) \qquad \mathrm{e}_{i,j} = -\sum_{l=k}^{i} q\delta(l,i)f_{j-i+l}(\Delta f_{j-i+l} + \Delta f_{i,j-i+l}) + \Delta f_j + \Delta f_{i,j} + \Delta C_{i,j}.$$

We may now calculate the elements of the variance–covariance matrix. The diagonal elements in the variance–covariance matrix corresponding to the $\mathrm{e}_{i,j}$ can be calculated as

$$\mathrm{Var}(\mathrm{e}_{i,j}) = \sum_{l=k}^{i-1} q^2 f_{j-i+l}^2(\sigma_y^2 + \sigma_a^2) - (qf_j - 1)(\sigma_y^2 + \sigma_a^2) + 1/4q^2 f_j^2(\sigma_y^2 + \sigma_a^2) + \sigma_c^2.$$

The covariance between two composite error terms $\mathrm{e}_{i,j}$ and $\mathrm{e}_{i',j'}$ depends on whether or not the two terms relate to the same cohort. If they come from two different cohorts, the age-year specific error term ($\Delta f_{i,j}$ or σ_a) does not contribute to their covariance. In calculating $\mathrm{Cov}(\mathrm{e}_{i,j}, \mathrm{e}_{i',j'})$, $j' \geq j$ can be assumed since the covariance–variance matrix is symmetrical.

The cohorts to which i year olds in year j and i' year olds in year j' belong, or to which the error terms $\mathrm{e}_{i,j}$ and $\mathrm{e}_{i',j'}$ refer,

© 1996 NRC Canada

are designated m_1 and m_2, where $m_1 = j - i + a$ and $m_2 = j' - i' + a$. Similarly, ages of first entry to the fishery are designated a_1 and a_2, where $a_1 = \max(1, i - j + 1)$ and $a_2 = \max(1, i' - j' + 1)$. Finally, the year when the two cohorts begin to be fished are designated by d_1 and d_2, where $d_1 = j - i + a_1$ and $d_2 = j' - i' + a_2$.

In calculating the covariances we can also assume that $m_1 \leq m_2$ because the covariance matrix is symmetric. This means that the first cohort m_1 enters the fishery first and that the two cohorts are fished simultaneously only from d_2 to j when $d_2 \leq j$. The covariances can now be obtained from the following equations.

(*i*) When $m_1 = m_2$, then the two composite error terms $e_{i,j}$ and $e_{i',j'}$ are from the same cohort, in which case

$$\mathrm{Cov}(e_{i,j}, e_{i',j'}) = \sum_{t=a_1}^{i-1} q^2 f_{j-i+t}^2(\sigma_a^2 + \sigma_y^2) + 1/2q^2 f_j^2(\sigma_a^2 + \sigma_y^2) - qf_j(\sigma_a^2 + \sigma_y^2).$$

(*ii*) When $m_1 < m_2$ and $j \leq j'$, if $d_2 \leq j$, then the two cohorts have not been fished simultaneously, in which case

$$\mathrm{Cov}(e_{i,j}, e_{i',j'}) = 0.$$

(*iii*) When $m_1 < m_2$ and $j \leq j'$, if $d_2 > j$, then the two cohorts have been fished simultaneously from year d_2 to year j, in which case

$$\mathrm{Cov}(e_{i,j}, e_{i',j'}) = \sum_{t=d_2}^{j-1} q^2 f_t^2 \sigma_y^2 + 1/2\delta(j,j')q^2 f_j^2 \sigma_y^2 - qf_j \sigma_y^2.$$

Estimation of catchability, natural mortality, and cohort size

When the assumed error structure is included, ordinary least squares regression breaks down, necessitating a more complicated estimation procedure than that used by Paloheimo (1980).

Given a catch data set with age i ranging from 1 to $a + 1$ and year j ranging from 1 to $n + 1$, there are altogether $(a + 1)(n + 1) - 2 = s$ data points available for estimation (data points at $a + 1$ years old in year 1 and at 1 year old in year $n + 1$ are excluded, Paloheimo 1980). Assuming that selectivities are constant among ages and catchabilities among years, the total number of parameters to be estimated is $(n + a - 1) + 1 + 1 = n + a + 1$ representing number of cohort sizes, q, and M. The two-dimensionally indexed catch data are replaced by a singly subscripted catch, thus, $y_r = \ln(O_{i,j})$, for $r = 1, \ldots, s$. The coding of doubly subscripted data into singly subscripted data follows the pattern shown in Table 1. The totality of log catches is denoted by a vector $\mathbf{y} = (y_1, \ldots, y_s)^T$, where the superscript T denotes the transpose. The parameters to be estimated are denoted by a vector $\mathbf{B}$, given as $\mathbf{B} = (\ln(qN_1), \ldots, \ln(qN_{n+a-1}), q, M)^T$, or in more generic notation as $\mathbf{B} = (b_1, \ldots, b_k, \ldots, b_p)^T$, where $p = n + a + 1$. Estimates of $\mathbf{B}$ are obtained by iteration. The current values of $\mathbf{B}$ are denoted by $\mathbf{B}$ and the new value by $\mathbf{B} + \Delta\mathbf{B}$, where $\Delta\mathbf{B} = (\Delta b_1, \ldots, \Delta b_p)^T$.

We rewrite eq. 3 as $y_r = g_r(\mathbf{B}) + e_r$. The set of predicted log catches is denoted by $\mathbf{g}(\mathbf{B}) = (g_1(\mathbf{B}), \ldots, g_s(\mathbf{B}))^T$. The covariance matrix of errors is denoted $\mathbf{V}$. We now choose the parameter values to minimize

$$(5) \quad (\mathbf{y} - \mathbf{g}(\mathbf{B}))^T \mathbf{V}^{-1} (\mathbf{y} - \mathbf{g}(\mathbf{B})).$$

While this is essentially an errors-in-variables problem we have chosen to solve it by the least squares technique with a parameter-dependent variance–covariance matrix. While in many cases the two methods agree there is no guarantee in a general case that the estimates will have reasonable statistical properties. As a check we have applied the method to simulated data with known parameter values.

If the covariances were zero, eq. 5 would be the weighted sum of the squared differences between the observed and predicted values. However, they are not and the problem is a weighted nonlinear least squares estimation problem where the variance–covariance matrix $\mathbf{V}$ also depends on one of the parameters to be estimated, namely q. There are general computer programs that can be used to find a solution for the above minimization problem; typically they require initial or starting values that are not too far from the actual solution. Such starting values may be obtained by replacing $\mathbf{V}$ with an identity matrix and estimating values of $\ln(qN_m)$ and q and M from eq. 5 (with $\mathbf{V} = \mathbf{I}$). This is essentially what was done in the earlier paper (Paloheimo 1980).

Rather than leaving the estimation to be carried out by some canned program we have written our own. To that end the difference between the observed and predicted y_r is denoted by $\Delta y_r = y_r - g_r(\mathbf{B})$. The set of differences is denoted by the vector $\Delta\mathbf{y} = (\Delta y_1, \ldots, \Delta y_s)^T$. The estimation procedure is based on the first order Taylor series expansion of the vector $\mathbf{g}$ and the matrix $\mathbf{V}$. The expansion for $\mathbf{B}$ is

$$(6) \quad g_r(\mathbf{B} + \Delta\mathbf{B}) = g_r(\mathbf{B}) + \left(\frac{\partial g_r(\mathbf{B})}{\partial \mathbf{B}}\right)^T \Delta\mathbf{B},$$

where the derivative is with respect to the vector $\mathbf{B}$. The expansion for $\mathbf{V}^{-1}$ evaluated at $q + \Delta q$ is

$$(7) \quad \mathbf{V}^{-1}(q + \Delta q) = \mathbf{V}^{-1} - \mathbf{V}^{-1}\,\dot{\mathbf{V}}_q\,\mathbf{V}^{-1}\,\Delta q,$$

where all the terms in $\mathbf{V}^{-1}\,\dot{\mathbf{V}}_q\,\mathbf{V}^{-1}$ are evaluated at q and where $\dot{\mathbf{V}}_q$ is the derivative of $\mathbf{V}$ with respect to q, i.e., $\dot{\mathbf{V}}_q = \partial\mathbf{V}/\partial q$. Equation 7 is derived in the Appendix. When eqs. 6 and 7 are substituted in eq. 5 the minimization is reduced to finding the roots of

$$(8) \quad \frac{\partial}{\partial \Delta B}\left((\Delta\mathbf{y} - \frac{\partial \mathbf{g}(\mathbf{B})}{\partial \mathbf{B}}\Delta\mathbf{B})^T(\mathbf{V}^{-1} + \mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\Delta q)\left(\Delta\mathbf{y} - \frac{\partial \mathbf{g}(\mathbf{B})}{\partial \mathbf{B}}\Delta\mathbf{B}\right)\right) = 0.$$

This, although it may not look it, is linear and can be solved for $\Delta\mathbf{B}$ (see Appendix). The value of $\mathbf{B}$ is then replaced by $\mathbf{B} + \Delta\mathbf{B}$ and the cycle of calculations is repeated until we obtain a convergence.

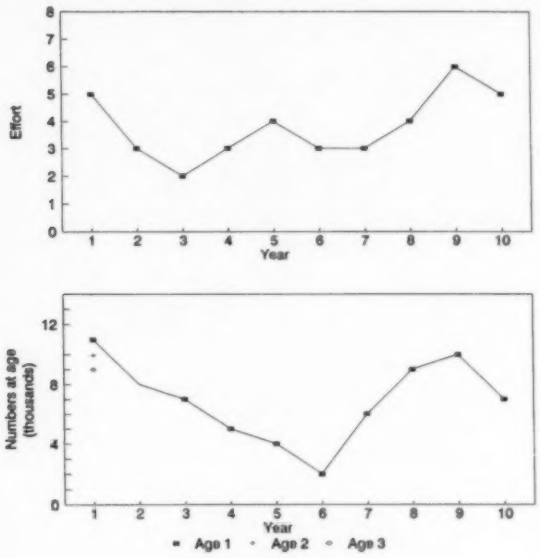
Simulation study

Observed efforts and true cohort sizes at recruitment were generated somewhat arbitrarily for a hypothetical fishery over a 10-year span as shown in Fig. 1. The catchability coefficient and natural mortality rate were assumed to be 0.1 and 0.2, respectively. Fishing mortality for the hypothetical fishery ranged from 0.2 to 0.6, typical of many mature fisheries in the world (Hilborn and Walters 1992; Parsons 1993). The cohort sizes at ages 2 and 3 in year 1 were taken to be the same as at age 1 in year 1, but subject to additional natural mortality.

The effective or true efforts were simulated by applying

© 1996 NRC Canada

Fig. 1. Observed efforts and cohort sizes used in the simulation runs.



log-normal errors to the observed efforts as in eq. 1 and true catch-at-age data were then calculated from the catch equation. The observed catch-at-age data were simulated by applying log-normal errors to the true catch-at-age data according to the error structure defined in eq. 1. Eighteen sets of errors for simulating the effort and catch data are defined in Table 2.

The simulated observed catch and effort data were used in estimating parameters using three different variance–covariance scenarios. In the first case we set $\mathbf{V} = \mathbf{I}$, which is the early model (Paloheimo 1980) and will be referred to as EVS (equal variance scenario hereafter). The true variance scenario (TVS) assumes that the true variances of the errors, σ_y, σ_a, and σ_c, are used in calculating the variance–covariance matrix $\mathbf{V}$, whereas the wrong variance scenario (WVS) assumed constant values of the error variances set at $\sigma_y = 0.1$, $\sigma_a = 0.05$, and $\sigma_c = 0.1$ in all cases. Thus, comparing the results from these last two scenarios will indicate whether the estimated parameters were sensitive to the error variances assumed in the estimation process.

To measure the extent to which the parameter estimates $\hat{b}_{i,k}$ (i refers to an estimate such as $\ln(qN_m)$, q, and M and k refers to the kth simulation) differ from the true values, the following indices were computed on the basis of 100 simulation runs: (*i*) mean estimated value

$$\bar{b}_i = \frac{1}{100}\sum_{k=1}^{100} \hat{b}_{i,k}$$

and (*ii*) mean squared error (MSE)

$$\text{MSE}\ (b_i) = \frac{1}{100}\sum_{k=1}^{100} (\hat{b}_{i,k} - b_i)^2.$$

MSE can be decomposed into a sum of the squared bias and the variance about the observed mean,

Table 2. Errors used in simulation.

Set	σ_y^*	σ_a	σ_c
1	0.05	0.05	0.10
2	0.05	0.10	0.10
3	0.05	0.20	0.10
4	0.10	0.05	0.10
5	0.10	0.10	0.10
6	0.10	0.20	0.10
7	0.20	0.05	0.10
8	0.20	0.10	0.10
9	0.20	0.20	0.10
10	0.05	0.05	0.20
11	0.05	0.10	0.20
12	0.05	0.20	0.20
13	0.10	0.05	0.20
14	0.10	0.10	0.20
15	0.10	0.20	0.20
16	0.20	0.05	0.20
17	0.20	0.10	0.20
18	0.20	0.20	0.20

Note: Because $\Delta f_{i,j}$ values were chosen independently without requiring $\Sigma_i \Delta f_{i,j} = 0$, the actual σ_y^2 is in fact given by $\sigma_y^2 + \sigma_a^2/(a+1)$ and σ_a^2 by $\sigma_a^2 a/(a+1)$.

$$\text{MSE}\ (b_i) = (\bar{b}_i - b_i)^2 + \frac{1}{100}\sum_{k=1}^{100} (\hat{b}_{i,k} - \bar{b}_i)^2.$$

Standard errors (SE) of each estimated parameter were also calculated in a manner similar to methods used in the linear regression formula (see Appendix).

Results

In our simulation there are altogether 13 parameters: 11 cohort sizes, M, and q. The cohorts N_1, N_2, N_{10}, and N_{11} are all based on either 2 or 3 years of data as opposed to 4 years for the rest of the cohorts. N_1 and N_2 are estimates of the cohort sizes at ages 3 and 2 whereas the rest (N_3 to N_{11}) are estimated at age 1. In what follows we look only at the estimate of the cohort sizes at age-1 entry to the fishery, i.e., the cohorts N_3 to N_{11}.

MSE and the biases of M, q and the average cohort size (actually the average of the values pertaining to $\ln(qN_m)$) are summarized in Figs. 2 and 3. For cohort sizes, only the average bias and the average MSE of the nine cohorts N_3 to N_{11} are given; the reason for limiting ourselves to the average is apparent later. In each figure we have calculated the average bias or MSE at each level of σ_y, σ_a, or σ_c. Since there were three levels of σ_y and σ_a and two levels of σ_c (Table 2), all combinations being replicated, the average of each level of σ_y and σ_a is based on six terms whereas the average of each of the two levels of σ_c is based on nine terms. Before graphing, the data were sorted in ascending order of the parameter being plotted.

We note from Figs. 2 and 3 that there are very little differences between the true variance (TVS) and wrong variance (WVS) scenarios. Apparently what matters is the pattern of the variance–covariance matrix rather than the specific numerical values. The estimates obtained using the equal variance scenario (EVS, i.e., the earlier model) have consistently higher

© 1996 NRC Canada

Fig. 2. Mean MSE of M, q, and av.ln(qN) for the different variance scenarios at each error level. See text for further explanations.

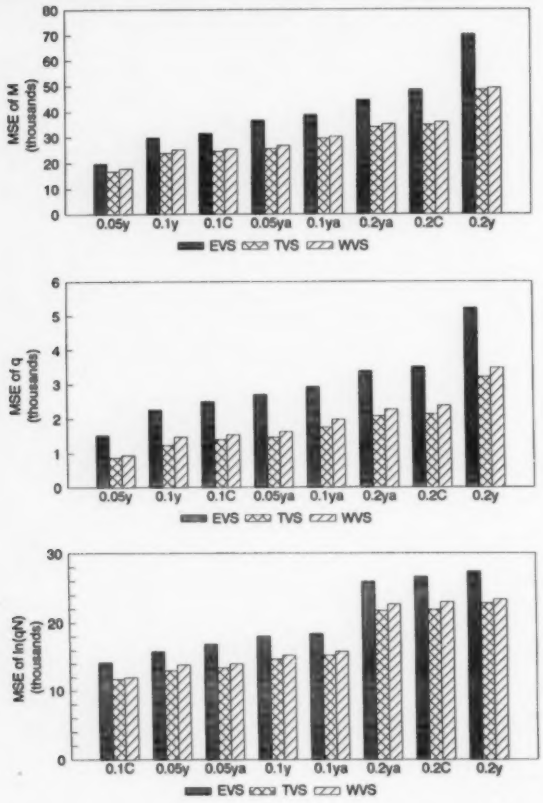
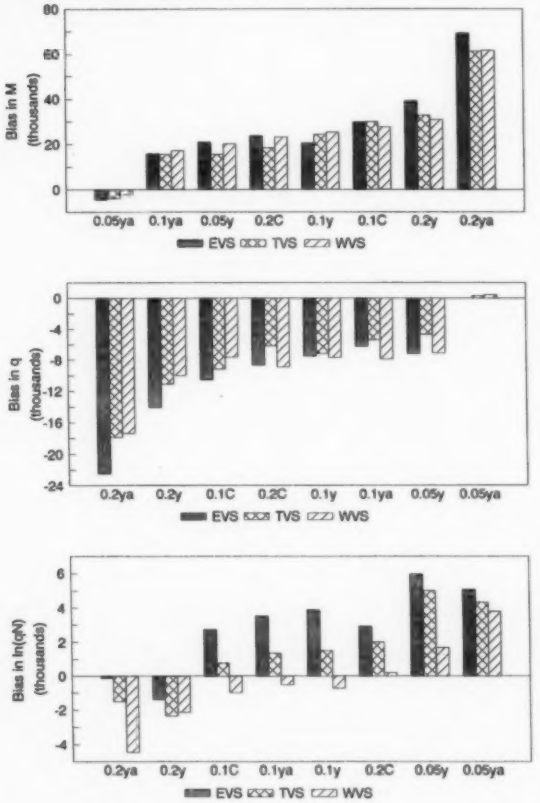

MSE of M (thousands)
0.05y 0.1y 0.1C 0.05ya 0.1ya 0.2ya 0.2C 0.2y
EVS TVS WVS
MSE of q (thousands)
0.05y 0.1y 0.1C 0.05ya 0.1ya 0.2ya 0.2C 0.2y
EVS TVS WVS
MSE of ln(qN) (thousands)
0.1C 0.05y 0.05ya 0.1y 0.1ya 0.2ya 0.2C 0.2y
EVS TVS WVS

Fig. 3. Mean bias in M, q, and av.ln(qN) for the different variance scenarios at each error level. See text for furter explanations.


Bias in M (thousands)
0.05ya 0.1ya 0.05y 0.2C 0.1y 0.1C 0.2y 0.2ya
EVS TVS WVS
Bias in q (thousands)
0.2ya 0.2y 0.1C 0.2C 0.1y 0.1ya 0.05y 0.05ya
EVS TVS WVS
Bias in ln(qN) (thousands)
0.2ya 0.2y 0.1C 0.1ya 0.1y 0.2C 0.05y 0.05ya
EVS TVS WVS

levels of bias and MSE than those obtained using either TVS or WVS, although the differences are not that large (about 40%).

Each simulation produced 100 estimates of each of the 13 parameters. The distribution of these estimates did not differ significantly from a normal random variable on the basis of the Shapiro–Wilk test for normality (Proc Univariate programs, SAS Institute Inc., Cary, N.C.). Thus, MSE and the bias completely characterize the distribution of the estimates as being normal with the estimated mean equal to the bias and the variance equal to the MSE – bias^2.

The smallest MSE occurs (Fig. 2) at $\sigma_y = 0.05$ followed by $\sigma_y = 0.1$ except for av.ln(qN), which has the smallest error (MSE) at $\sigma_c = 0.1$ followed by $\sigma_y = 0.05$. The largest MSE is found at $\sigma_y = 0.2$ and the second largest at $\sigma_c = 0.2$. Biases (Fig. 3) are generally low and do not exhibit as clear-cut a relationship with the input error levels as does MSE. Whether the observed biases were significantly different from zero was tested by calculating the t value as

$$t = \frac{\text{bias}}{((\text{MSE} - \text{bias}^2)/99)^{1/2}}$$

For M and q these are distributed as Student's t with 99 degrees of freedom. For av.ln(qN) the distribution of the t values above is not clear, since the estimates of different ln(qN_m) values may be correlated. It turns out that the biases of M and q are not significantly different from zero except at $\sigma_a = 0.2$. We have no explanation as to why variability between ages would cause bias in the estimates. For what it is worth, none of the t values of av.ln(qN) calculated by the above equation were significantly different from zero.

MSE can be accurately predicted from the input error levels. To arrive at a linear predictor we note first that the actual between-year error level, simulated by drawing random numbers from $N(0,\sigma_y^2)$, is in fact higher than σ_y for the following reason. To each observed effort f_j we added $\Delta f_j + \Delta f_{i,j}$. This random component can also be written as

$$\Delta f_j + \frac{\sum_{i=1}^{a+1} \Delta f_{i,j}}{a+1} + \Delta f_{i,j} - \frac{\sum_{i=1}^{a+1} \Delta_{i,j}}{a+1} .$$

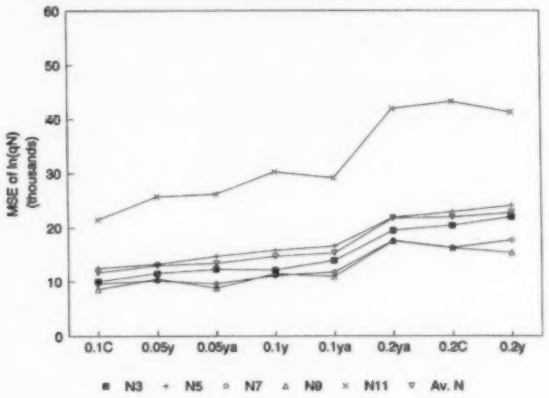
From this we can see that the actual between-year error variance is $\sigma_y^2 + \sigma_a^2/(a+1)$ and the actual within-year, between-age variance is $\sigma_a^2\, a/(a+1)$. Linear predictors of MSE, obtained by regressing MSE values on the actual or true error levels, are tabulated in Table 3 showing the additive contributions of each type of input or data error level. The linear predictor explains better than 95% of the variability in all cases.

© 1996 NRC Canada

Table 3. Regression coefficients of MSE on the input data error levels.

Parameter	σ_c	σ_a	σ_y	Constant	r^2
M	0.103	0.055	0.216	−0.017	0.98
q	0.007	−0.001	0.018	−0.002	0.95
Av.ln(qN)	0.102	0.042	0.076	−0.013	0.99

Fig. 4. Mean MSE of $\ln(qN_m)$ for $m = 3, 5, 7, 9$, and 11 and the mean MSE of av.ln(qN) for the different variance scenarios at each error level. See text for further explanations.



The magnitude of the coefficients can be used to assess the sensitivity of MSE to errors in the different input data. From Table 3 we see that σ_a has the least effect on the MSE of M, q, and av.ln(qN). The effect of σ_c is about twice as large as that of σ_a on the MSE of M, q, and av.ln (qN), while the effect of σ_y is about four times as large as that of σ_a on the MSE of M and q but about the same as that on the MSE of av.ln (qN).

While a linear function of σ_a, σ_y, and σ_c explains most of the variability in MSE we also tested the significance of the higher order terms. At the error levels of σ_y, σ_a, and σ_c that were used in our simulations the only second order term that was significant was σ_y^2. Nevertheless, this suggests that as the error in the basic data increases beyond the values used in this study the estimates degenerate very rapidly.

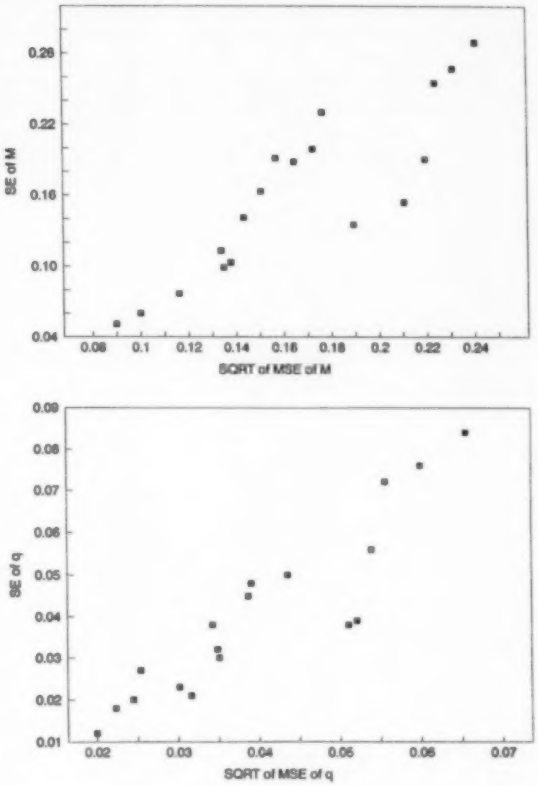
In Fig. 4 we show the MSEs for $\ln(qN_3)$, $\ln(qN_5)$, . . ., $\ln(qN_{11})$ and for the average of $\ln(qN_3)$, . . ., $\ln(qN_{11})$ using TVS. The MSEs are very much the same for all cohorts except for N_{11}, which understandably has the highest MSE. The graphs using the other variance scenarios show a similar pattern, with little difference in the MSE or in the bias between cohorts other than N_{11}. This is our justification for not giving separate tables for each cohort.

We also calculated SE for each estimate at each simulation run. The average SEs and the square root of the MSEs are closely correlated (Fig. 5) with r^2 values of 0.71 for M and 0.78 for q, and with slopes in both cases of about 1. The close correspondence suggests that SEs can in fact be used to calculate confidence limits for the estimated parameters.

Discussion

The cohort-based regression model (Paloheimo 1980) has been criticized for lacking a satisfactory error structure by Fournier and Archibald (1982), Deriso et al. (1985), and perhaps others. While this is true, all the other methods we know of are similarly constrained. For instance, both Fournier and Archibald (1982) and Deriso et al. (1985) minimize the sums of squares of the differences between the observed and predicted log catches-at-age. These differences are not independent but correlated as shown here. Our eq. 5 is essentially the sums of squares of these differences corrected for dependencies among the data points.

Fig. 5. The square root of MSE for each simulation set plotted against the standard error (mean over the 100 runs) returned by the estimation algorithm.



Incorporation of what perhaps might be an acceptable error structure makes the algorithm for solving eq. 5 (TVS or WVS) much more complex than a linear regression or EVS procedure and usually requires three or four iterations. There is an improvement in the accuracy of the estimated parameters, although the improvement is not all that great. In almost all cases the MSEs of the estimates using EVS were less than 40% larger than those obtained by TVS or WVS. There were only minor differences in the results obtained using TVS and WVS. This makes application of the model possible, since it is almost impossible to estimate the error variance in the input data.

The estimates of q and M at the highest error levels used in our simulation runs ($\sigma_y = 0.2$ or $\sigma_c = 0.2$) had MSE values high enough to make the estimates not significantly different from zero. Moreover, at these levels in about 30% of the runs the

© 1996 NRC Canada

iterations failed to converge (although EVS always converged). Since these iterations were discarded and new simulations run, the reported MSEs are actually underestimates of the true MSEs associated with these levels of σ_y or σ_c. In some cases (10 out of 30) the convergence was obtained by arbitrarily reducing the values in the variance–covariance matrix, a practice that is probably justifiable because the estimates are in general not sensitive to the actual values in **V**.

While the error structure could perhaps still be improved, the major limitation of the present model is, we believe, the validity of assumed relationship between observed efforts and the resultant fishing mortality rate, or between f_j and E_{ij}. Prior to application of the present method to any real data set one should apply our earlier model (Paloheimo and Chen 1993) for estimating the annual fishing mortalities. While the procedure requires an estimate of M (and only M!), even when an erroneous value is used, the resultant fishing mortalities are highly correlated with the true efforts and hence can offer guidance on the use of the present method.

Acknowledgments

We gratefully acknowledge the contributions of a thorough review of the first draft of the manuscript by Grant G. Thompson of the Alaska Fisheries Science Center. The manuscript was greatly improved because of his comments. This study was financially supported by a Natural Sciences and Engineering Research Council operating grant to J.E.P. and an Ontario Graduate Scholarship to Y.C.

References

Bard, Y. 1974. Nonlinear parameter estimation. Academic Press, New York.

Crecco, V., and Overholz, W.I. 1990. Causes of density-dependent catchability for Georges Bank haddock *Melanogrammus aeglefinus*. Can. J. Fish. Aquat. Sci. **47**: 385–394.

Deriso, R.B., Quinn, T.J., II, and Neal, P.R. 1985. Catch-age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. **42**: 815–824.

Doubleday, W.G. 1976. A least squares approach to analyzing catch at age data. Int. Comm. Northwest Atl. Fish. Res. Bull. **12**: 69–81.

Fournier, D., and Archibald, C.P. 1982. A general theory for analyzing catch at age data. Can. J. Fish. Aquat. Sci. **39**: 1195–1207.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Megrey, B.A. 1989. Review and comparison of age-structured stock assessment models from theoretical and applied points of view. Am. Fish. Soc. Symp. **6**: 8–48.

Paloheimo, J.E. 1980. Estimating mortality rates in fish populations. Trans. Am. Fish. Soc. **109**: 378–386.

Paloheimo, J.E., and Chen, Y. 1993. Estimating effective efforts from catch at age data. Can. J. Fish. Aquat. Sci. **50**: 2421–2428.

Paloheimo, J.E., and Dickie, M. 1964. Abundance and fishing success. Rapp. P.V. Reun. Cons. Int. Explor. Mer, **155**: 152–163.

Parsons, L.S. 1993. Management of marine fisheries in Canada. Can. Bull. Fish. Aquat. Sci. No. 225.

Pope, J.G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Int. Comm. Northwest Atl. Fish. Res. Bull. **9**: 65–74.

Pope, J.G., and Shepherd, J.G. 1982. A simple method for the consistent interpretation of catch-at-age data. J. Cons. Cons. Int. Explor. Mer, **40**: 176–184.

Appendix

To prove eq. 7 and to derive an algorithm to solve eq. 5 (or eq. 8) we will be using well-established rules for taking vector derivatives of matrices. Such rules are found in most texts on matrix algebra or in texts on nonlinear estimation (e.g., Bard 1974).

Derivation of eq. 7

We can write approximately

$$\mathbf{V}(q + \Delta q) = \mathbf{V}(q) + \dot{\mathbf{V}}_q(q)\Delta q$$

$$\mathbf{V}^{-1}(q + \Delta q) = \mathbf{V}^{-1}(q) + \dot{\mathbf{V}}_q^{-1}(q)\Delta q$$

where $\dot{\mathbf{V}}_q^{-1}$ stands for the derivative of $\mathbf{V}^{-1}$ with respect to q, evaluated at q. Multiplying the two above equations we get

$$\mathbf{I} = (\mathbf{V} + \dot{\mathbf{V}}_q\Delta q)(\mathbf{V}^{-1} + \dot{\mathbf{V}}_q^{-1}\Delta q)$$

$$= \mathbf{I} + \mathbf{V}\dot{\mathbf{V}}_q^{-1}\Delta q + \dot{\mathbf{V}}q\mathbf{V}^{-1}\Delta q$$

where the second order term has been ignored. The last equation gives us an expression for $\dot{\mathbf{V}}_q^{-1}$ that has been used in eq. 7.

Algorithm for solving eq. 5

We want to minimize

$$(\Delta\mathbf{y} - \frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B})^T(\mathbf{V}^{-1} - \mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\Delta q)(\Delta\mathbf{y} - \frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B})$$

with respect to $\Delta\mathbf{B}$. Note that $\partial\mathbf{g}/\partial\mathbf{B}$ is an $s \times p$ matrix defined as

$$\frac{\partial\mathbf{g}}{\partial\mathbf{B}} = \left(\frac{\partial g_i}{\partial b_j}\right), \quad i = 1, \ldots, s, j = 1, \ldots, p$$

We write the product of the matrices as $C - D$, where

$$C = \Delta\mathbf{y}^T\mathbf{V}^{-1}\Delta\mathbf{y} - \Delta\mathbf{y}^T\mathbf{V}^{-1}\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B} - (\partial\mathbf{B})^T\left(\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\right)^T\mathbf{V}^{-1}\Delta\mathbf{y} + (\Delta\mathbf{B})^T\left(\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\right)^T\mathbf{V}^{-1}\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B}$$

$$D = \Delta\mathbf{y}^T\mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\Delta\mathbf{y}\Delta q - \Delta\mathbf{y}^T\mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B}\,\Delta q - (\Delta\mathbf{B})^T\left(\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\right)^T\mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\Delta\mathbf{y}\Delta q + (\Delta\mathbf{B})^T\left(\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\right)^T\mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B}\,\Delta q$$

Taking derivatives of C and D with respect to $\Delta\mathbf{B}$ we get

$$\frac{\partial C}{\partial\Delta\mathbf{B}} = -2\left(\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\right)^T\mathbf{V}^{-1}\Delta\mathbf{y} + 2\left(\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\right)^T\mathbf{V}^{-1}\frac{\partial\mathbf{g}}{\partial\mathbf{B}}\Delta\mathbf{B}$$

and

$$\frac{\partial D}{\partial\Delta\mathbf{B}} = \Delta\mathbf{y}^T\mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\Delta\mathbf{y}(0,\ldots,1,0)^T - (c_1,\ldots c_{p-2},2c_{p-1},c_p)^T\Delta q$$

© 1996 NRC Canada

where $c_1, \ldots, c_p$ are elements of $2(\partial \mathbf{g}/\partial \mathbf{B})\mathbf{V}^{-1}\dot{\mathbf{V}}_q\mathbf{V}^{-1}\Delta y$ and where we have ignored all the second order terms. Setting both $\partial \mathbf{C}/\partial \Delta \mathbf{B}$ and $\partial \mathbf{D}/\partial \Delta \mathbf{B}$ to zero and subtracting them we get

$$\Theta + \Gamma \Delta \mathbf{B} + (c_1, \ldots, c_{p-2}, 2c_{p-1}, c_p)^T \Delta q = 0$$

where

$$\Theta = \begin{pmatrix} \theta_1 \\ \cdots \\ \theta_p \end{pmatrix}$$

and

θ_i is the ith element of $-2\left(\frac{\partial \mathbf{g}}{\partial \mathbf{B}}\right)^T \mathbf{V}^{-1}\Delta \mathbf{y}$ for $i = 1, \ldots, p-2, p\ (i \neq p-1)$

$$\theta_{p-1} = (-2\left(\frac{\partial \mathbf{g}}{\partial \mathbf{B}}\right)^T \mathbf{V}^{-1}\ \Delta \mathbf{y})_{p-1} - \Delta \mathbf{y}^T\, \mathbf{V}^{-1}\, \dot{\mathbf{V}}_q\, \mathbf{V}^{-1} \Delta \mathbf{y}$$

$$\Gamma = (\gamma_{ij}) = (2\left(\frac{\partial \mathbf{g}}{\partial \mathbf{B}}\right)^T \mathbf{V}^{-1}\left(\frac{\partial \mathbf{g}}{\partial \mathbf{B}}\right)_{i,j}, \ i,j = 1, \ldots, p$$

If we write

$$\Phi = \begin{pmatrix} \gamma_{11} & \cdots & \gamma_{1,p-2} & \gamma_{1,p-1} + c_1 & \gamma_{1p} \\ \cdot & \cdots & \cdot & \cdot & \cdot \\ \gamma_{p-1,1} & \cdots & \gamma_{p-1,p-2} & \gamma_{p-1,p-1} + 2c_{p-1} & \gamma_{p-1,p} \\ \gamma_{p,1} & \cdots & \gamma_{p,p-2} & \gamma_{p,p-1} + c_p & \gamma_{p,p} \end{pmatrix}.$$

The whole expression can now be written as

$$\Theta + \Phi \Delta \mathbf{B} = 0$$

giving

$$\Delta \mathbf{B} = \Phi^{-1}\, \Theta$$

The standard errors of the estimates have been calculated from $(-\Phi^{-1})_{ii}\sigma$ where $(\Phi^{-1})_{ii}$ is the i*th* diagonal element of the inverse of Φ and σ is the residual sum of squares (eq. 5) divided by the number of degrees of freedom ($an - 2$).

© 1996 NRC Canada

Assessing the reliability of salinity inference models from diatom assemblages: an examination of a 219-lake data set from western North America

Susan E. Wilson, Brian F. Cumming, and John P. Smol

Abstract: Surface-sediment samples for diatom analyses, along with water chemistry and other limnological data, were collected from 113 fresh and saline lakes on the southern Interior Plateau and in the southern Rocky Mountain Trench of British Columbia, and combined with an existing calibration data set of 102 lakes from the southern interior of British Columbia and 11 saline lakes from the northern Great Plains. Diatom-based salinity inference models (= transfer functions) were developed using weighted-averaging and weighted-averaging with tolerance downweighting regression and calibration. The effects of species deletions and data-set size on the predictive ability (root mean square error of prediction after bootstrapping ($RMSE_{boot}$)) of the models were evaluated by developing models using different species deletion criteria and by varying the number of lakes in the data set. The study emphasizes the value of training sets with a large number of lakes and of taxa to develop transfer functions with robust and reliable estimates of species optima and tolerances and to provide more and closer modern analogues for inferring salinity from diatom assemblages downcore. The new salinity transfer function generated from this larger data set ($RMSE_{boot} = 0.37$, $r^2 = 0.87$) is an improvement over an earlier model developed from this region.

Résumé : Des échantillons de sédiments superficiels, destinés à l'analyse des diatomées ainsi qu'à celle de la chimie de l'eau et à la recherche d'autres données limnologiques, ont été prélevés dans 113 lacs d'eau douce et d'eau salée du Plateau intérieur du sud et du Sillon des Rocheuses du Sud en Colombie-Britannique, et les données ont été combinées à la série existante de données de calibration couvrant 102 lacs de l'intérieur du sud de la Colombie-Britannique et 11 lacs salés des Grandes Plaines boréales. Les modèles d'inférence (= fonctions de transfert) de la salinité à partir des diatomées ont été élaborés à partir d'une régression et d'une calibration faisant appel à la moyenne pondérée et à la moyenne pondérée avec sous-pondération de la tolérance. Nous avons évalué les effets du retrait des espèces et de la taille de la série de données sur la capacité prédictive ($RMSE_{boot}$) en élaborant des modèles faisant appel à différents critères de retrait des espèces et en faisant varier le nombre de lacs dans la série de données. L'étude met l'accent sur la valeur des bases d'apprentissage comptant un grand nombre de lacs et des taxons pour la formulation de fonctions de transfert comportant des estimations robustes et fiables des optima des espèces et des tolérances, et pour fournir des analogues modernes plus nombreux et plus proches en vue de déduire la salinité à partir des assemblages de diatomées dans la carotte. La nouvelle fonction de transfert de la salinité générée à partir de cette grande série de données ($RMSE_{boot} = 0{,}37$, $r^2 = 0{,}87$) constitue une amélioration par rapport à un modèle antérieur élaboré pour cette région.
[Traduit par la Rédaction]

Introduction

Changes in biological assemblages are a powerful and useful technique to detect environmental change. In the field of paleolimnology, quantitative inference techniques based on species assemblages of siliceous algae and invertebrates have now been used to address a variety of environmental issues including lake acidification, lake eutrophication, and climatic change (e.g., reviewed by Anderson 1993; Charles and Smol 1994; Smol 1995).

Accurate estimates of past environmental conditions based on biological assemblages assume that we can statistically model the present-day responses of species in relation to the environmental conditions under which the species live. The surface-sediment calibration approach is one of the most powerful methods for determining species–environment relationships for paleolimnological research. A surface-sediment calibration data set (also called a training set, sensu Birks et al. 1990) consists of a number of lakes that cover a diverse range of limnological conditions. From each study lake, the biological assemblages preserved in the surface sediments (e.g., top 1 cm, which represents the last few years of sediment accumulation) are identified and enumerated. These sediments provide an integrated sample, in space and time, of the taxa that have accumulated over the previous few years. Environmental data, consisting of present-day physical, chemical, and biological

Received August 28, 1995. Accepted February 15, 1996.
J13052

S.E. Wilson, B.F. Cumming, and J.P. Smol.[1] Paleoecological Environmental Assessment and Research Laboratory, Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada.

[1] Author to whom all correspondence should be addressed. e-mail: smolj@biology.queensu.ca

© 1996 NRC Canada

Fig. 1. Map of southern British Columbia showing the four sampling regions and the number of lakes in each region that together comprise the 224-lake data set.

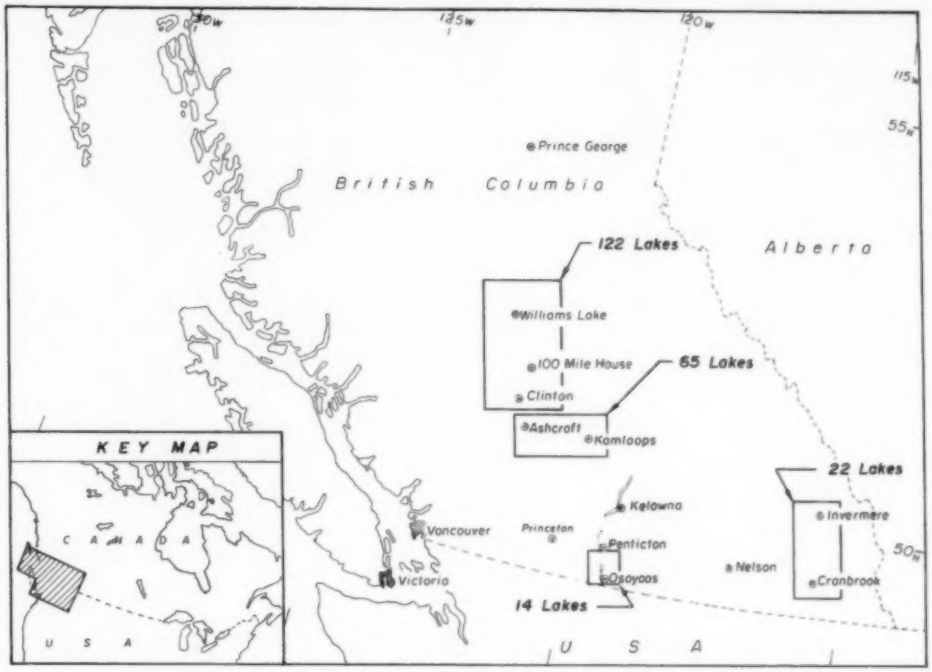


Table 1. The geology, vegetation, and general climate of the four regions sampled.

Region	Geology	Biogeoclimatic zones represented	Mean annual precipitation (cm·year $^{-1}$)	Mean daily July temperature (°C)	Mean daily January temperature (°C)
Cariboo–Chilcotin	Mainly flat-lying early Tertiary basaltic flows	Mainly interior Douglas-fir, some bunchgrass	<30–50	14–18	–5 to –15
Kamloops	Paleozoic sedimentary and volcanic rock formations with granitic intrusions, some flat to gently dipping early Tertiary lavas	Mainly ponderosa pine and bunchgrass, some interior Douglas-fir	<30–40	18–22	–5 to –10
Osoyoos–Penticton	Cretaceous – early Tertiary stratified and volcanic rocks; metamorphic rocks (mainly schists and gneisses) of unknown age	Ponderosa pine and bunchgrass, some interior Douglas-fir	<30–40	20–22	0 to –5
Cranbrook	Chiefly Quaternary sediments that include glacial deposits and extensive Holocene fluvial sediments	Ponderosa pine, interior Douglas-fir, some interior cedar–hemlock	30–50	18–20	–5 to –10

Note: Descriptions are taken from Farley (1979) and Valentine et al. (1978).

variables that are thought to be important, are also collected from each lake. A number of multivariate statistical techniques are then used to determine which environmental variables are most highly related to the biological assemblages (e.g., ter Braak and Prentice 1988; Reyment 1991; Shi 1993). Information extracted from calibration data sets can then be used quantitatively to infer past environmental conditions from the species composition in a sample, by use of a transfer function. This approach assumes taxonomic uniformitarianism between the surface-sediment and core samples (e.g., Birks et al. 1990; Charles and Smol 1994).

Recently, Wilson et al. (1994) published a diatom-based transfer function for inferring lake water salinity for lakes from the interior region of British Columbia, based on a training set of 102 lakes. This transfer function was derived to provide a tool to infer high-resolution changes in effective moisture from appropriate closed-basin lakes in regions of British Columbia and the Canadian prairies. Although the predictive ability of the salinity transfer function was strong (root mean square error of prediction after bootstrapping ($RMSE_{boot}$) = 0.42, r^2 = 0.82) and appropriate for reconstructing past lake water salinities from fossil diatom assemblages in many lake sediment

© 1996 NRC Canada

Table 2. Location, late-summer salinity, and sampling depth of the 113 lakes added to the British Columbia training set.

Lake code	Lake name	Latitude (N)	Longitude (W)	Late-summer salinity ($g \cdot L^{-1}$)	Sampling depth (m)
BC1	Monroe	49°21.9′	115°51.9′	0.02	18.0
BC2*	Pattons	49°32.8′	115°46.7′	2.11	0.3
BC3	Mineral	49°20.9′	115°51.2′	0.10	24.0
BC4	Horseshoe	49°34.4′	115°31.0′	0.09	10.5
BC5	Alkaline	49°30.3′	115°29.8′	9.28	3.0
BC6	"Flat tire"	49°42.8′	115°40.7′	5.59	2.0
BC7	Wasa	49°46.7′	115°43.9′	0.20	8.5
BC8	Lazy	49°49.6′	115°37.2′	0.11	7.5
BC9*	Sylvan	49°31.1′	115°43.4′	5.10	0.3
BC10*		49°31.7′	115°43.1′	8.14	0.3
BC11	Campbell	49°38.9′	115°38.7′	0.37	6.0
BC12	Topaz	50°50.0′	116°24.2′	0.16	4.0
BC13	Lillian	50°30.2′	116°06.0′	0.32	12.0
BC14	Johnson	49°57.4′	115°45.9′	0.50	6.0
BC15	Premier	49°55.7′	115°39.0′	0.13	11.0
BC16	Hahas	49°44.9′	115°49.4′	0.45	10.5
BC17	McGinty	49°41.2′	115°51.5′	2.53	0.3
BC18	Moyie	49°21.8′	115°50.0′	0.02	37.0
BC19	Tamarack	49°55.3′	115°48.3′	0.57	4.0
BC20	Larchwood	49°57.0′	115°47.8′	0.33	8.0
BC21	Horn	49°19.2′	119°43.6′	0.19	30.0
BC22	Green "South"	49°18.2′	119°34.2′	2.15	17.0
BC23	Mahoney	49°17.4′	119°34.9′	23.46	16.0
BC24	Kilpoola	49°01.6′	119°33.8′	9.12	6.5
BC25	Blue	49°02.4′	119°33.7′	16.61	14.0
BC26	Frank	49°01.7′	119°39.1′	0.90	6.5
BC27	Spotted	49°05.0′	119°34.0′	371.00	0.5
BC28	Ripley	49°14.6′	119°37.9′	0.12	12.0
BC29	Madden	49°13.9′	119°37.5′	0.12	21.0
BC30	Burnell	49°12.6′	119°36.7′	0.48	8.0
BC31		49°04.3′	119°34.2′	25.82	2.0
BC32	Deadman	49°05.7′	119°33.3′	3.46	2.0
BC33	Tugulnuit	49°12.0′	119°32.4′	0.15	7.5
BC34	Vaseaux	49°17.4′	119°31.8′	0.11	12.0
BC35	Fish	52°06.6′	122°33.8′	0.18	8.5
BC36		52°06.9′	122°32.9′	12.26	4.0
BC37		52°05.9′	122°31.5′	12.57	1.5
BC38	Twin	52°04.2′	122°29.2′	1.26	2.5
BC39		52°03.5′	122°28.4′	15.57	1.0
BC40	Goose	52°02.9′	122°27.7′	1.23	2.0
BC41	Lost	52°02.6′	122°27.8′	3.29	1.0
BC42**	Long	52°01.7′	122°27.3′	5.06	3.5
BC43**	Barnes	52°00.5′	122°27.6′	10.06	4.0
BC44**	Lye	52°00.8′	122°29.4′	6.55	4.5
BC45	Drummond	52°02.2′	122°32.3′	3.86	4.5
BC46**	Roundup	52°02.1′	122°30.5′	12.34	2.0
BC47	"Soap"	52°24.8′	122°27.8′	0.48	5.5
BC48	Yorston	52°18.0′	122°22.9′	0.30	2.5
BC49	Coyote	52°16.2′	122°20.9′	2.47	0.4
BC50**	"McMurray"	52°16.0′	122°24.3′	6.07	1.5
BC51	Forest	52°17.8′	122°01.7′	0.28	9.0
BC52	McLeese	52°25.3′	122°18.1′	0.11	44.0
BC53	Cuisson	52°29.2′	122°18.7′	0.11	7.0
BC54	Rimrock	52°29.0′	122°19.2′	0.11	12.0
BC55	Parker	51°53.5′	121°46.5′	0.40	1.0
BC56	Dingwall	51°51.3′	121°45.3′	0.21	3.5
BC57		51°52.7′	121°41.8′	15.88	1.0
BC58	Felker	51°57.0′	121°59.9′	0.26	10.5

© 1996 NRC Canada

Table 2 (*concluded*).

Lake code	Lake name	Latitude (N)	Longitude (W)	Late-summer salinity ($g \cdot L^{-1}$)	Sampling depth (m)
BC59	Chimney	51°55.2′	121°58.0′	0.28	14.0
BC60	Bobtail	51°53.7′	121°47.7′	0.64	1.0
BC61	Island	51°53.5′	121°49.3′	0.57	1.0
BC62	McIntosh	52°08.9′	121°28.1′	0.06	17.0
BC63	Miner	52°10.3′	121°32.4′	0.04	2.5
BC64	Spokin	52°10.9′	121°45.9′	0.09	5.5
BC65	Dewar	52°11.8′	121°51.9′	0.36	4.0
BC66	Big	52°23.0′	121°50.6′	0.13	31.0
BC67	Whiffle	52°28.1′	121°13.0′	0.06	6.0
BC68	Ussa	52°25.6′	121°18.6′	0.05	14.0
BC69	Nikwit	52°25.4′	121°16.1′	0.06	11.5
BC70	Helena	51°47.3′	121°38.0′	0.25	6.5
BC71	Whitehorse	51°50.0′	121°23.1′	0.12	3.0
BC72	Rail	51°56.8′	121°28.0′	0.08	9.0
BC73	Irish	51°32.9′	121°10.1′	0.39	7.0
BC74	Little Green	51°26.2′	121°03.2′	0.53	2.0
BC75	Watch	51°27.8′	121°06.2′	0.13	2.0
BC76	Little Horse	51°27.3′	121°09.1′	0.46	2.0
BC77	Green "North"	51°25.3′	121°12.2′	0.53	20.0
BC78*		51°18.1′	121°21.9′	3.18	0.5
BC79	Horse	51°35.3′	121°07.1′	0.09	~24
BC80	Valentine	51°35.2′	121°26.9′	0.32	4.5
BC81*	Big	51°40.1′	121°27.0′	0.37	6.0
BC82*	Watson	51°42.6′	121°20.7′	0.71	2.0
BC83*	Last Chance	51°19.6′	121°38.5′	93.07	0.1
BC84	Alberta	51°21.2′	121°38.3′	4.89	0.5
BC85	White	51°23.9′	121°53.2′	4.93	10.5
BC86	Meadow	51°21.8′	121°45.5′	1.70	2.0
BC87	Big Bar	51°18.9′	121°48.8′	0.15	15.0
BC88*	Little White	51°17.1′	121°41.2′	3.70	1.5
BC89	Trurans	51°13.8′	121°35.3′	0.10	2.5
BC90		51°20.5′	121°18.6′	1.51	1.5
BC91		50°39.7′	120°30.7′	19.45	1.0
BC92**		50°39.9′	120°30.7′	26.50	1.0
BC93**	Polygon Pond	50°40.1′	120°27.6′	69.04	2.0
BC94		50°40.1′	120°29.4′	15.52	1.5
BC95	Chuwhels	50°35.0′	120°35.7′	0.05	8.5
BC96	Dominic	50°35.2′	120°40.6′	0.08	22.0
BC97	Durand	50°36.9′	120°39.9′	0.09	2.5
BC98	Face	50°32.6′	120°38.0′	0.05	7.5
BC99	Lac Le Jeune	50°28.8′	120°29.2′	0.13	23.5
BC100	Ironmask	50°39.1′	120°27.1′	364.30	0.3
BC101	Peter Hope	50°17.8′	120°18.7′	0.16	~26
BC102	Stump	50°21.7′	120°22.2′	0.90	19.0
BC103	Palmer Meadows	50°22.9′	120°10.8′	0.09	3.0
BC104	Roche	50°28.4′	120°09.2′	0.11	11.5
BC105	Marquart	50°04.7′	120°39.5′	0.27	20.0
BC106	Venables	50°34.2′	121°20.6′	2.29	0.5
BC107*		50°35.5′	121°20.4′	620.29	0.0
BC108	Barnes	50°42.3′	121°14.3′	0.06	11.5
BC109	North Barriere	51°20.0′	119°50.3′	0.02	44.0
BC110	Scott	51°13.5′	120°17.5′	0.05	6.5
BC111	Dunsapie	51°11.0′	120°20.0′	0.06	3.0
BC112	Cleland	50°50.0′	116°23.3′	0.84	~27
BC113	Upper Brady	50°40.3′	116°15.0′	1.56	12

Note: Lake names in quotation indicate unofficial names.
*Low diatom abundance (100–200 valves counted).
**No diatoms were found in the surface sediments.

© 1996 NRC Canada

cores, there are a number of lakes for which this salinity transfer function is inadequate because many of the species assemblages from the cores are distinct from those present in the calibration lakes (e.g., many intervals from Mahoney Lake, British Columbia (S.E Wilson, unpublished data), the early Holocene of Clearwater Lake, Saskatchewan (Wilson 1996), and the early Holocene of U60, Yukon (R. Pienitz, Université de Laval, unpublished data). Given that only slightly more than 100 sites have been sampled, and there are hundreds of taxa, it is not surprising that we have encountered analogue problems. Many of the taxa identified in the current calibration data sets are too rare to be included in the transfer functions, but they have been found in higher abundances in sediment cores. Furthermore, because the number of species in a diatom-based transfer function is frequently greater than the number of calibration lakes, estimates of the ecological characteristics of many taxa (e.g., salinity optima) are based on limited data. In summary, larger calibration data sets should increase the number of taxa in the transfer functions, increase our understanding of the ecological characteristics of all taxa, and provide more analogues appropriate for downcore reconstructions.

Since the Wilson et al. (1994) publication, surface-sediment samples for diatom analyses and water chemistry data were collected from an additional 113 fresh and saline lakes from British Columbia. Additionally, 11 saline lakes with the diatom *Cyclotella caspia* (now recognized as *Cyclotella choctawhatcheeana* Prasad. (Carvalho et al. 1995) were added from the northern Great Plains (NGP) region (Fritz et al. 1993), as this diatom was rarely encountered in the expanded British Columbia data set, but is an extremely abundant taxon in at least one of our lake cores from British Columbia. This resulted in a surface-sediment calibration data set of 219 lakes from which a new salinity inference model was developed. This paper examines the influence of species deletion criteria and number of lakes on the salinity transfer function and addresses the following questions that are pertinent to other calibration data sets: (*i*) How do we decide what taxa should be included in the transfer function? (*ii*) How many lakes are required to produce a stable transfer function with the lowest possible RMSE of prediction?

Study region

Of the 113 lakes added to our data set, 91 are located on the southern Interior Plateau of British Columbia, whereas the remaining 22 lakes are situated in the southern Rocky Mountain Trench near Cranbrook (Fig. 1). In general, these regions are in a semiarid to subhumid climate, with differences in the geology, vegetation, temperature, and amount of precipitation (Table 1). The coordinates of the new 113 lakes from British Columbia along with the late-summer salinities are shown in Table 2. The location, chemistry, and physical characteristics of the original 102 lakes can be found elsewhere (Wilson et al. 1994; Cumming et al. 1995). The names, locations, and salinities of the 11 lakes incorporated from the NGP data set are given in Table 3. Additional characteristics of these lakes and a description of their surroundings can be found in Fritz et al. (1993).

Materials and methods

Surface-sediment samples were collected from the additional 113 British Columbia lakes in August and September of 1993 using a Glew (1991) miniature gravity corer. The cores were taken from near the center of each lake, and the upper 1 cm of sediment was removed for diatom analyses. A homogenized sediment subsample from each lake was placed in a 20-mL glass vial and 10% HCl was added to remove any carbonates. The samples were left undisturbed for 24 h before the remaining HCl was removed by aspiration. The vials were then placed in a support rack and a 50:50 mixture of concentrated nitric and sulphuric acid was slowly added up to the one-half or three-quarter mark of each vial. The samples were stirred and left for at least 24 h before being placed in a water bath (~90°C) for 3 h. Each day the samples were aspirated down to 1 cm depth above the sediment, topped up with distilled water, stirred, and allowed to settle for at least 24 h, until all acid was removed (about six washes). A portion of the resulting slurry was then pipetted, in a series of dilutions, on to cover slips and allowed to evaporate. The dried cover slips were then mounted on glass slides using Hyrax® mounting medium (refractive index = 1.71).

Diatoms were identified and enumerated using either a Leitz Dialux 20 microscope (1250× magnification with differential interference contrast (DIC) optics, numerical aperture = 1.32 or a Leitz DMRB microscope (1000× magnification with DIC optics, numerical aperture = 1.30). A minimum of 300 diatom valves (usually over 400) was counted, except where diatoms were absent (7 lakes) or sparse (9 lakes) (Table 2). The diatom slides from the initial 111 calibration lakes and those from the NGP region were recounted at this time to ensure consistent taxonomy. In the initial 111 lakes, 9 were without diatoms and 14 had low diatom abundances (Wilson et al. 1994); however, with recounting, four of these low abundance lakes (C15, C28, C31, C58) had their diatom counts brought to above 300 valves. One lake from the NGP data set had low diatom abundances (only 100 valves were counted from Stink Lake). *Chaetoceros* cysts and valves were enumerated as outlined in Wilson et al. (1994). Diatom nomenclature followed sources listed in Wilson et al. (1994), and the majority of taxa encountered are documented in Cumming et al. (1995).

Water chemistry analyses for Na, K, Mg, Ca, Cl, dissolved inorganic carbon (DIC), SO_4, pH, and conductivity followed protocols outlined in Wilson et al. (1994) and Cumming et al. (1995), with the following exceptions. Oxygen readings were taken using a Hydrolab multiparameter water-quality data transmitter instead of a YSI model oxygen meter and probe. In most lakes, conductivity, temperature, and pH profiles were taken with the Hydrolab in addition to the YSI model 33 S-C-T conductivity and temperature profiles, and hand-held electronic pH meter readings. The hand-held pH meters were calibrated prior to use at each lake. Salinity was calculated as the sum of Na, K, Ca, Mg, DIC, SO_4, and Cl concentrations.

Salinity models presented here were based on weighted-averaging (WA) and weighted-averaging with tolerance downweighting ($WA_{(tol)}$) regression and calibration (Birks et al. 1990), using the computer program WACALIB version 3.3 (Line et al. 1994). Inverse regression was used in the deshrinking step, and the estimated species tolerances were adjusted using Hill's (1973) N2 diversity measure. The $RMSE_{boot}$, given in log salinity units, was used to assess the predictive abilities of the salinity inference models (e.g., Birks et al. 1990; Birks 1994; Wilson et al. 1994). The bootstrap error has two components: (*i*) s_1 being that part of the prediction error owing to estimation error in the individual taxon parameters (the optimum in WA, and the optimum and tolerance in $WA_{(tol)}$) used in the calibration step and (*ii*) s_2 being that part of the error owing to natural variation in diatom assemblages under similar environmental conditions (e.g., two lakes could have the same salinity value but have different diatom assemblages; Birks et al. 1990; Birks 1994). The models were based on late-summer salinity calculations from the British Columbia lakes and midsummer salinity calculations from the NGP lakes (Fritz et al. 1993).

© 1996 NRC Canada

Table 3. Names, locations, and salinities of the 11 lakes added from the northern Great Plains data set (Fritz et al. 1993).

Lake name	Location	Salinity ($g \cdot L^{-1}$)
Basin	Saskatchewan, Canada	21.21
Big Quill	Saskatchewan, Canada	37.91
Deadmoose	Saskatchewan, Canada	21.50
East Devil's	North Dakota, U.S.A.	9.90
Free People	North Dakota, U.S.A.	8.92
George	North Dakota, U.S.A.	21.24
Medicine	South Dakota, U.S.A.	38.58
Redberry	Saskatchewan, Canada	18.92
Sayer	Saskatchewan, Canada	18.75
Stink	North Dakota (Stutsman County), U.S.A.	26.68
Waldsea	Saskatchewan, Canada	20.48

Note: All of these lakes contained the diatom *Cyclotella choctawhatcheeana*, which was not abundant in the surface sediments of the British Columbia lakes, but does occur in some Holocene lake sediments from the region.

Sampling protocols used for lakes in the NGP region are outlined in Fritz et al. (1993), with the main differences being that water chemistry analyses and sediment collection were undertaken in midsummer, and the upper 3 cm of surface sediment was used to represent the present-day diatom assemblage.

A weighted averaging partial least squares (WA-PLS) (ter Braak et al. 1993) salinity model was also developed, but it produced a higher RMSE of prediction after jackknifing than the simpler WA models and therefore is not presented.

Results and discussion

Salinity inference models: determining species deletion criteria

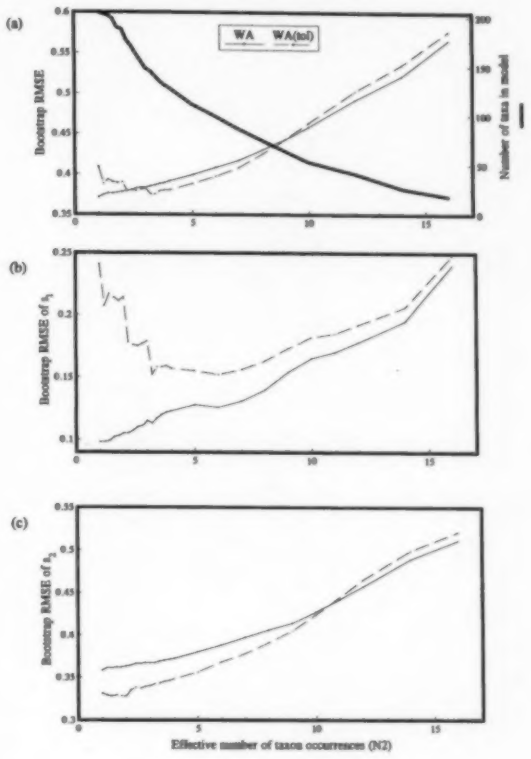
A total of 219 lakes, from the initial 235 lakes sampled, were used in the development of salinity inference models owing to an absence of diatoms in the surface sediments of 16 British Columbia lakes (see Table 2 in the present study and Appendix I in Wilson et al. 1994).

Over 400 diatom taxa were identified from the 219 lakes; however, the species list was modified to include only those taxa having relative abundances of ≥1% in at least 2 lakes, reducing the number to 204 (Table 4).

Virtually all diatom calibration data sets published to date have determined their criteria for species deletion in an ad hoc manner (e.g., having 1% relative abundance in at least one lake and present in two or more lakes (e.g., Gasse et al. 1995; Hall and Smol 1992; Birks et al. 1990), 1% abundance in at least one lake (e.g., Fritz et al. 1993), 2% abundance in at least one lake (e.g., Fritz et al. 1991), and 1% abundance in at least three lakes (e.g., Wilson et al. 1994)). The choice of various cut-off criteria may affect the predictive abilities of different models (e.g., WA vs. $WA_{(tol)}$). In an effort to provide some guidelines for deletion of species from data sets being used to develop transfer functions, we assessed the influence of selected species deletion criteria on WA and $WA_{(tol)}$ salinity inference models by varying the effective number of species occurrences (Hill's N2) required for individual taxa to be included in the inference models. By using an increasing N2 as a species deletion criterion, those taxa whose parameters (optima and tolerances) are determined by a limited number of lakes are eliminated first, followed by those with more stable estimates because they are determined from a larger number of lakes. For example, if a taxon occurs in five lakes at a relative abundance of 50% in one lake and <1% in the other lakes (N2 ~ 1), its species parameters would be largely determined by one lake, whereas if a taxon occurred in five lakes at an abundance of 50% in each lake (N2 = 5), its parameters would be determined equally from those five lakes. Taxa were eliminated according to their effective number of occurrences (given in Table 4), such that when the deletion criterion was set at N2 = 1, no further taxa were eliminated from the initial list of 204, whereas at N2 = 5, all taxa having an N2 of less than 5 were eliminated from the calibration models (Fig. 2*a*).

The lowest $RMSE_{boot}$ of prediction occurred in the WA model when all 204 taxa were included ($RMSE_{boot}$ = 0.37, N2 = 1; Fig. 2*a*). The $WA_{(tol)}$ model performed as well as or better than WA when the N2 deletion criterion was between 2.2 and 9, achieving its lowest $RMSE_{boot}$ (0.38) at N2 around 3 (Fig. 2*a*). However, the $WA_{(tol)}$ model never achieved a lower $RMSE_{boot}$ than the lowest WA model with an N2 of 1. These results corroborate the findings of Birks (1994), who

Fig. 2. Plot showing variations in (*a*) the bootstrap root mean square error of prediction ($RMSE_{boot}$), (*b*) the $RMSE_{boot}$ of s_1, and (*c*) the $RMSE_{boot}$ of s_2 for weighted-averaging regression and calibration (WA) and weighted-averaging with tolerance downweighting regression and calibration $WA_{(tol)}$, as the effective number of occurrences (N2) required for diatom taxa to be included in the diatom salinity inference model is increased.



© 1996 NRC Canada

Table 4. Estimates of taxon optima and tolerances, presented as upper and lower salinity limits, as in Wilson et al. (1994), for those taxa having ≥1% relative abundance in at least 2 of the 219 lakes.

Taxon name and authority	No. of occurrences	N2	Maximum relative abundance (%)	Salinity optimum ($g \cdot L^{-1}$)	Lower limit ($g \cdot L^{-1}$)	Upper limit ($g \cdot L^{-1}$)
Achnanthes conspicua A. Mayer 1919	24	8.7	4.9	0.10	0.02	0.58
Achnanthes exigua Grun. in Cleve & Grun. 1880	8	4.8	1.7	0.11	0.05	0.26
Achnanthes lanceolata ssp. *frequentissima* Lange-Bertalot 1991	13	9.9	1.6	0.13	0.02	0.89
Achnanthes minutissima Kütz. 1833	114	34.9	36.9	0.38	0.01	12.39
Achnanthes pusilla Grun. in Cleve & Grun. 1880	2	1.9	2.0	0.02	0.02	0.02
Achnanthes rosenstockii Lange-Bertalot 1991	11	4.7	4.6	0.14	0.06	0.33
Achnanthes subatomoides (Hust.) Lange-Bertalot & Archibald in Krammer & Lange-Bertalot 1985	2	1.4	4.6	0.02	0.02	0.02
Achnanthes ziegleri Lange-Bertalot 1991	32	20.8	2.8	0.13	0.03	0.58
Amphora acutiuscula Kütz. 1844	43	16.0	28.4	52.12	4.84	561.05
Amphora coffeaeformis (Agardh) Kütz. 1844	24	9.3	8.6	7.93	0.96	65.61
Amphora libyca Ehrenb. 1840	70	8.3	91.8	0.36	0.07	1.75
Amphora cf. *pediculus* (Kütz.) Grun. ex A. Schmidt 1875	51	19.7	13.9	0.20	0.01	2.59
Amphora subcapitata (Kisselev) Hust. 1959	11	4.4	7.1	2.39	0.51	11.14
Amphora thumensis (A. Mayer) Krieger 1929	16	7.3	4.4	0.13	0.04	0.43
Amphora veneta Kütz. 1844	39	7.7	47.6	1.79	0.30	10.86
Amphora sp. 1 PISCES	22	9.6	23.8	9.42	1.47	60.26
Amphora sp. 2 PISCES	21	3.7	50.9	2.58	0.61	10.96
Amphora sp. 3 PISCES	5	3.4	2.5	3.26	1.19	8.89
Amphora sp. 4 PISCES	19	5.4	24.6	33.42	5.52	202.30
Amphora sp. 5 PISCES (in Cumming et al. 1995, Plate 41, Figs. 13 and 14)	3	1.7	24.2	1.25	1.04	1.50
Amphora sp. 6 PISCES	4	3.8	2.8	2.70	0.68	10.74
Anomoeoneis sphaerophora (Ehrenb.) Pfitz. 1871	19	10.1	4.2	1.55	0.11	22.86
Anomoeoneis sphaerophora fo. 1 PISCES	60	24.3	82.0	4.23	0.82	21.68
Anomoeoneis sphaerophora fo. 2 PISCES	28	12.0	99.0	17.66	1.87	166.34
Anomoeoneis sphaerophora fo. *costata* (Kütz.) Schmid 1977	63	19.1	47.8	2.07	0.26	16.63
Asterionella formosa Hass. 1850	72	20.6	75.5	0.15	0.02	0.90
Aulacoseira ambigua (Grun. in Van Heurck) Simonsen 1979	13	4.3	23.8	0.09	0.01	0.71
Aulacoseira distans (Ehrenb.) Simonsen 1979	4	1.4	22.9	0.02	0.01	0.03
Aulacoseira granulata (Ehrenb.) Simonsen 1979	2	1.2	12.9	0.11	0.09	0.13
Aulacoseira subarctica (O.Müll.) Haworth 1988	13	2.0	46.0	0.42	0.03	5.77
Brachysira aponina Kütz.	4	2.1	15.8	31.55	15.31	65.01
Brachysira vitrea (Grun.) Ross in Hartley 1986	38	12.5	19.7	0.36	0.03	4.52
Campylodiscus clypeus (Ehrenb.) Ehrenb. ex Kütz. 1844	13	6.4	4.7	7.28	1.64	32.21
Chaetoceros muelleri Lemm. 1898	23	9.0	63.3	17.91	3.67	87.30
Cocconeis neothumensis Krammer 1991	17	12.5	1.2	0.19	0.02	1.59
Cocconeis pediculus Ehrenb. 1838*		4.3	17.3	20.23	9.16	44.67
Cocconeis placentula cf. var. *placentula* Ehrenb. 1838	21	14.5	2.2	0.32	0.02	5.96
Cocconeis placentula var. *euglypta* (Ehrenb.) Grun. 1884	32	12.5	9.3	0.35	0.02	5.20
Cocconeis placentula cf. var. *lineata* (Ehrenb.) Van Heurck 1880–1885	12	8.5	1.7	0.29	0.03	2.80
Craticula accomoda (Hust.) D.G. Mann in Round et al. 1990	9	3.2	5.8	0.32	0.04	2.76
Craticula ambigua (Ehrenb.) D.G. Mann in Round et al. 1990	35	11.2	28.4	1.74	0.15	19.59
Craticula cuspidata (Kütz.) D.G. Mann in Round et al. 1990	29	6.5	15.5	0.76	0.07	8.24
Craticula cuspidata fo. 1 PISCES	7	2.1	6.7	4.52	0.57	36.06
Craticula cuspidata fo. 2 PISCES	9	5.2	5.0	2.21	0.12	40.83
Craticula halophila (Grun. ex Van Heurck) D.G. Mann in Round et al. 1990 fo. 1 PISCES	25	4.9	55.7	0.73	0.10	5.47
Craticula halophila fo. 2 PISCES	77	20.1	36.3	1.00	0.08	12.56
Craticula halophila fo. 3 PISCES	18	9.8	98.3	3.71	1.02	13.52
Cyclostephanos cf. *delicatus* (Genkal) Kling & Håkansson 1990	6	2.7	18.8	0.14	0.02	0.93
Cyclostephanos cf. *invisitatus* (Hohn & Hellerman) Theriot, Stoermer & Håkansson 1987	9	5.0	19.1	0.19	0.03	1.36
Cyclostephanos cf. *tholiformis* Stoermer, Håkansson, & Theriot 1987	9	4.7	19.5	0.11	0.03	0.52
Cyclotella bodanica var. aff. *lemanica* (O.Müll. ex Schröt.) Bachm. 1903 (includes var. *lemanica*)	65	16.8	56.2	0.24	0.03	1.82

© 1996 NRC Canada

Table 4 (*continued*).

Taxon name and authority	No. of occurrences	N2	Maximum relative abundance (%)	Salinity optimum ($g \cdot L^{-1}$)	Lower limit ($g \cdot L^{-1}$)	Upper limit ($g \cdot L^{-1}$)
Cyclotella choctawhatcheeana Prasad. 1990	17	7.9	22.4	20.28	5.14	79.98
Cyclotella comensis Grun. in Van Heurck 1882	8	3.1	22.4	0.20	0.06	0.63
Cyclotella glomerata Bachm. 1911	6	2.4	28.0	0.03	0.00	0.41
Cyclotella meneghiniana Kütz. 1844	27	4.3	26.1	1.37	0.06	30.20
Cyclotella michiganiana Skvort. 1937	30	6.6	50.0	0.99	0.05	18.32
Cyclotella quillensis Bailey 1922*	4	1.7	5.9	4.78	0.21	109.90
Cyclotella stelligera (Cleve & Grun. in Cleve) Van Heurck 1882 (includes *pseudostelligera*)	15	2.6	37.8	0.03	0.00	1.10
Cymbella cf. *angustata* (W.Sm.) Cleve 1894	28	12.1	6.6	0.70	0.13	3.78
Cymbella cesatii (Rabenh.) Grun. in A. Schmidt 1881 (includes cf. *cesatii*)	9	3.5	6.1	0.25	0.07	0.87
Cymbella cistula (Ehrenb. in Hemp. & Ehrenb.) Kirchn. 1878	24	12.8	3.9	0.50	0.07	3.67
Cymbella cf. *cymbiformis* Agardh 1830	4	2.8	1.2	0.28	0.10	0.81
Cymbella cymbiformis var. *nonpunctata* Font. 1917	34	12.2	19.6	0.72	0.14	3.74
Cymbella delicatula Kütz. 1849	7	3.3	5.6	0.15	0.05	0.48
Cymbella leptoceros (Ehrenb.) Kütz. 1844	7	2.1	7.0	0.13	0.02	0.69
Cymbella microcephala Grun. in Van Heurck 1880	40	19.0	8.2	0.27	0.03	2.51
Cymbella cf. *microcephala*	8	6.3	2.4	0.35	0.06	1.99
Cymbella minuta Hilse ex Rabenh. 1862	30	15.9	2.7	0.33	0.01	10.07
Cymbella muelleri Hust. 1938 (includes fo. *ventricosa*)	6	4.6	1.2	0.46	0.23	0.91
Cymbella pusilla Grun. ex A. Schmidt 1875	66	24.8	99.0	5.47	0.33	89.54
Cymbella silesiaca Bleisch ex Rabenh. 1864	19	2.5	26.9	0.67	0.12	3.57
Cymbella sp. 1 PISCES	32	9.6	29.7	1.01	0.10	9.91
Cymbella sp. 2 PISCES	6	2.6	5.2	0.40	0.20	0.77
Cymbella sp. 3 PISCES (in Cumming et al. 1995, Plate 45, Figs. 7–9)	9	3.8	4.8	0.19	0.03	1.50
Cymbella sp. 4 PISCES (in Cumming et al. 1995, Plate 48, Fig. 5)	5	2.7	3.7	0.59	0.19	1.83
Denticula kuetzingii Grun. 1862	40	5.0	64.5	0.23	0.03	1.91
Denticula valida (Pedicino) Grun. in Van Heurck 1881	5	3.6	3.4	9.46	0.28	316.23
Diatoma tenue Agardh 1812	4	1.1	32.3	13.06	7.05	24.21
Diatoma tenue var. *elongatum* Lyngb. 1819	11	3.8	3.9	2.22	0.19	25.59
Entomoneis paludosa (W.Sm.) Reimer in Patr. & Reimer 1975	25	11.3	5.7	2.42	0.17	34.43
Epithemia adnata (Kütz.) Bréb. 1838	23	1.7	59.8	1.09	0.30	3.91
Epithemia argus (Ehrenb.) Kütz. 1844	20	6.4	8.7	0.95	0.13	6.90
Fragilaria brevistriata Grun. in Van Heurck 1885	78	18.6	79.6	0.19	0.02	1.65
Fragilaria brevistriata var. *papillosa* Cleve-Euler 1953	11	7.2	2.5	0.06	0.03	0.12
Fragilaria capucina complex	38	8.5	19.7	0.26	0.04	1.48
Fragilaria capucina var. *gracilis* (Østr.) Hust. 1950	7	5.3	3.0	0.09	0.02	0.43
Fragilaria capucina var. *mesolepta* (Rabenh.) Rabenh. 1864	22	2.9	66.8	0.37	0.03	3.99
Fragilaria construens (Ehrenb.) Grun. 1862	44	10.6	60.0	0.08	0.03	0.22
Fragilaria construens var. *binodis* (Ehrenb.) Grun. 1862	9	3.4	5.8	0.10	0.03	0.37
Fragilaria construens var. *venter* (Ehrenb.) Grun.in Van Heurck 1881	48	14.2	32.7	0.13	0.01	2.42
Fragilaria crotonensis Kitt. 1869	47	14.5	53.5	0.17	0.02	1.45
Fragilaria cyclopum (Brutschy) Lange-Bertalot 1980	23	12.0	4.7	0.11	0.03	0.37
Fragilaria famelica (Kütz.) Lange-Bertalot 1980	12	5.1	7.3	7.40	0.14	384.59
Fragilaria fasciculata (Agardh) Lange-Bertalot 1980	21	8.8	11.6	8.93	0.44	181.55
Fragilaria lapponica Grun. in Van Heurck 1881	12	7.8	1.9	0.19	0.01	3.10
Fragilaria nanana Lange-Bertalot 1991 (in Cumming et al. 1995, Plate 8, Figs. 10–14)	35	7.1	36.5	0.36	0.02	5.83
Fragilaria cf. *neoproducta* Lange-Bertalot 1991	2	2.0	1.2	0.22	0.08	0.62
Fragilaria parasitica (W.Sm.) Grun. in Van Heurck 1881	13	10.4	1.4	0.12	0.03	0.50
Fragilaria pinnta Ehrenb. 1843	74	15.1	93.8	0.10	0.01	0.66
Fragilaria cf. *pinnata*	38	14.0	27.3	0.10	0.01	0.78
Fragilaria pinnata var. *intercedens* (Grun. in Van Heurck) Hust. 1931	9	4.7	3.6	0.11	0.01	1.56
Fragilaria pinnata var. *lancettula* (Schum.) Hust. in A. Schmidt 1913	10	3.5	4.3	0.25	0.01	4.84
Fragilaria pulchella (Ralfs ex Kütz.) Lange-Bertalot 1980	23	13.2	3.0	15.49	1.85	130.02
Fragilaria cf. *tenera* (W.Sm.) Lange-Bertalot 1980	44	16.9	14.4	0.32	0.01	8.07
Fragilaria ulna complex	22	12.4	2.3	0.42	0.04	4.31

© 1996 NRC Canada

Table 4 (*continued*).

Taxon name and authority	No. of occurrences	N2	Maximum relative abundance (%)	Salinity optimum ($g \cdot L^{-1}$)	Lower limit ($g \cdot L^{-1}$)	Upper limit ($g \cdot L^{-1}$)
Fragilaria ulna var. *acus* (Kütz.) Lange-Bertalot 1980 (includes cf. *ulna* var. *acus*)	40	9.4	18.8	0.34	0.04	3.10
Fragilaria sp. 1 PISCES	10	4.0	7.7	0.10	0.02	0.49
Gomphonema acuminatum Ehrenb. 1832	7	3.0	4.3	0.18	0.06	0.56
Gomphonema angustum Agardh 1831	28	14.6	5.4	0.29	0.04	1.99
Gomphonema aff. *angustum* Agardh 1831	5	2.3	3.3	0.34	0.05	2.33
Gomphonema cf. *aquaemineralis* Lange-Bertalot & Reichardt	6	3.7	2.9	1.30	0.07	24.60
Gomphonema gracile Ehrenb. 1838	15	5.5	9.7	0.19	0.01	2.43
Gomphonema parvulum (Kütz.) Kütz. 1849	21	10.1	7.9	0.29	0.02	3.33
Gomphonema sp. 1 PISCES (includes cf. sp. 1)	26	8.5	12.8	0.78	0.06	10.67
Gomphonema sp. 2 PISCES (in Cumming et al. 1995, Plate 43, Figs. 8–12)	4	2.4	5.8	0.49	0.07	3.25
Hantzschii amphioxys (Ehrenb.) Grun. 1877	14	2.2	15.5	4.62	0.15	139.64
Mastogloia elliptica (Agardh) Cleve ex A. Schmidt 1893	19	6.5	10.5	20.65	4.36	97.95
Mastogloia smithii Thwaites ex W.Sm. 1856 (includes var. *lacustris*)	26	7.3	15.0	0.89	0.03	30.90
Navicula bulnheimii Grun. in Van Heurck 1880	11	4.8	44.0	27.99	9.27	84.53
Navicula capitata Ehrenb. 1838	9	7.1	1.6	0.42	0.00	94.84
Navicula capitata var. *hungarica* (Grun.) Ross 1947	10	6.8	1.1	0.83	0.01	111.94
Navicula cf. *cincta* (Ehrenb.) Ralfs in Pritch. 1861 (in Cumming et al. 1995, Plate 20, Fig. 11)	8	5.2	2.7	1.05	0.02	49.55
Navicula aff. *cincta* (form in between cf. *cincta* & aff. *digitoradiata* var. *minima*)	11	2.1	43.8	16.22	1.51	174.58
Navicula cf. *crucialis* (O.Müll.) Freng. 1929	3	1.4	80.3	21.78	12.53	37.84
Navicula cryptocephala Kütz. 1844	16	4.9	11.8	0.09	0.03	0.30
Navicula cryptotenella Lange-Bertalot 1985	42	21.8	5.9	0.18	0.03	0.96
Navicula cryptotenella fo. 1 PISCES	25	6.6	24.2	0.25	0.04	1.48
Navicula cf. *cryptotenella*	6	2.5	8.3	0.15	0.02	1.10
Navicula aff. *digitoradiata* var. *minima* Cleve-Euler 1953	28	10.2	15.6	63.68	2.59	620.29
Navicula diluviana Krasske 1933	14	6.2	6.9	0.12	0.04	0.34
Navicula gallica var. *laevissima* (Cleve) Lange-Bertalot 1985*	4	3.2	3.8	20.14	17.30	23.44
Navicula laevissima Kütz. 1844	29	13.2	5.7	0.21	0.05	0.82
Navicula libonensis Schoeman 1970	27	8.0	9.3	0.63	0.05	7.55
Navicula minima Grun. in Van Heurck 1880 (includes cf. *minima*)	31	8.2	15.6	0.10	0.01	0.79
Navicula cf. *minuscula* Grun. in Van Heurck 1880	7	2.1	6.2	0.09	0.02	0.41
Navicula mutica Kütz. 1844	8	1.7	9.7	5.79	0.59	56.89
Navicula oblonga (Kütz.) Kütz. 1844	55	15.3	33.5	0.47	0.08	2.61
Navicula cf. *pavillardii* Hust. 1939*	4	2.1	6.5	20.32	14.26	28.97
Navicula pseudoventralis Hust. 1953	18	13.3	2.0	0.09	0.03	0.22
Navicula pupula Kütz. 1844	50	4.8	74.7	0.38	0.06	2.43
Navicula radiosa Kütz. 1844	16	6.4	4.4	0.20	0.04	1.00
Navicula submuscula Manguin 1941	6	2.7	4.5	0.35	0.01	17.34
Navicula cf. *subtilissima* Cleve 1891	2	1.6	6.4	0.49	0.22	1.12
Navicula trivialis Lange-Bertalot 1980	6	3.3	6.8	0.13	0.06	0.29
Navicula cf. *utermoehlii* Hust. 1943	23	10.4	8.6	0.10	0.02	0.48
Navicula veneta Kütz. 1844	55	13.7	55.3	2.71	0.16	45.81
Navicula aff. *veneta*	45	16.5	16.0	2.57	0.16	42.07
Navicula vitabunda sensu Krammer & Lange-Bertalot 1986 non Hust. 1930	17	9.3	4.4	0.10	0.04	0.26
Navicula sp. 1 PISCES	7	2.5	27.1	2.25	0.14	36.56
Navicula sp. 4 PISCES	4	2.0	5.7	0.09	0.02	0.52
Navicula sp. 5 PISCES	15	7.1	10.0	0.20	0.04	1.05
Navicula sp. 6 PISCES fo. 2	16	5.9	82.2	235.50	42.46	620.29
Navicula sp. 7 PISCES	3	2.0	67.9	363.92	248.31	533.33
Neidium ampliatum sensu Krammer & Lange-Bertalot 1985	13	5.5	4.8	0.53	0.10	2.96
Nitzschia amphibia Grun. 1862	49	10.3	30.7	0.58	0.04	8.59
Nitzschia amphibia fo. *frauenfeldii* (Grun.) Lange-Bertalot 1987 (in Cumming et al. 1995, Plate 36, Fig. 14)	12	8.5	1.7	0.49	0.08	3.11
Nitzschia aurariae Chol. 1966	4	1.1	47.3	14.79	2.00	109.65

© 1996 NRC Canada

Table 4 (*concluded*).

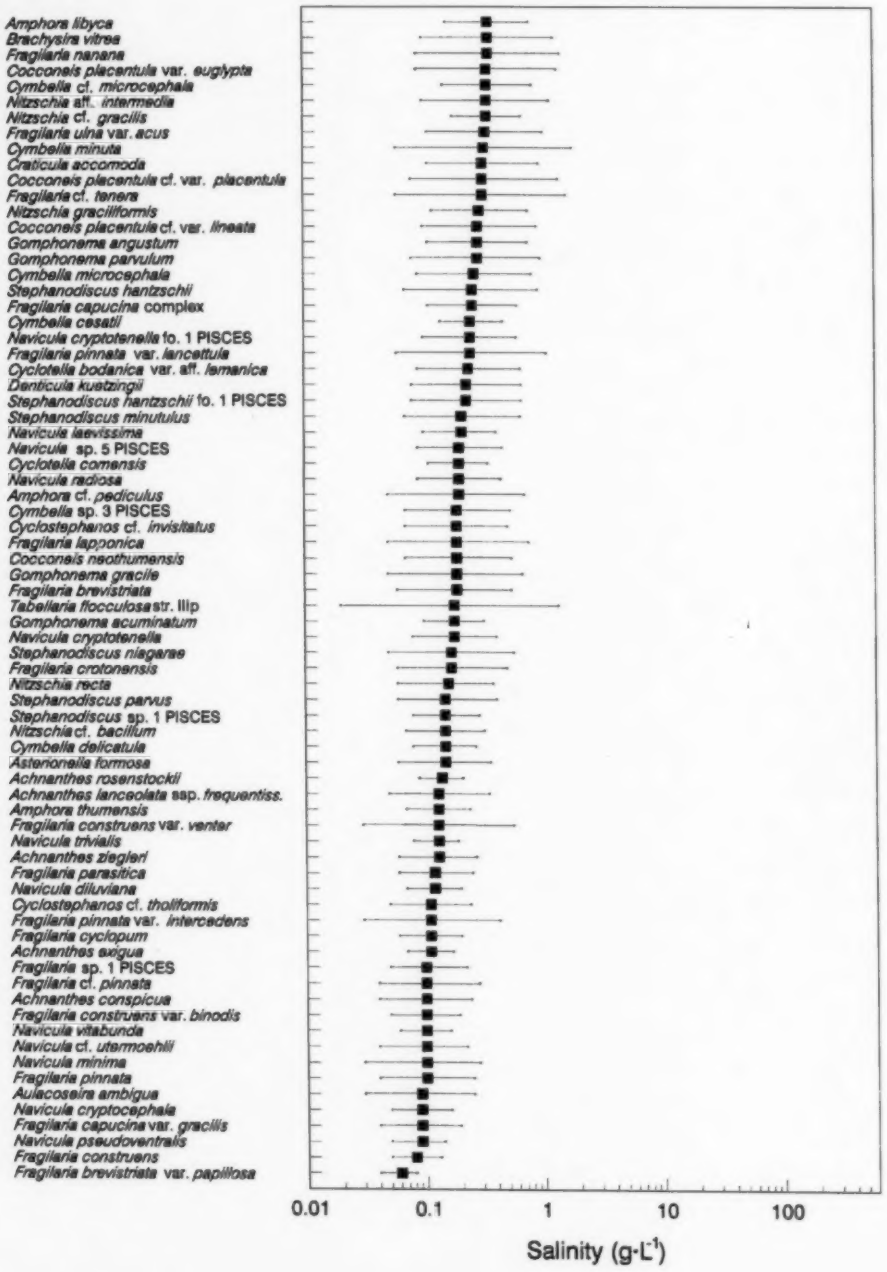
Taxon name and authority	No. of occurrences	N2	Maximum relative abundance (%)	Salinity optimum ($g \cdot L^{-1}$)	Lower limit ($g \cdot L^{-1}$)	Upper limit ($g \cdot L^{-1}$)
Nitzschia cf. *bacillum* Hust. in A. Schmidt et al. 1922	16	8.2	4.3	0.15	0.03	0.68
Nitzschia cf. *bergii* Cleve-Euler 1953	9	1.7	29.1	9.57	0.41	222.33
Nitzschia cf. *communis* Rabenh. 1860	25	8.7	21.6	69.02	2.71	620.29
Nitzschia draveillensis Coste & Ricard 1980 (= *N. acicularis* in Wilson et al. 1994)	16	2.8	10.1	0.20	0.03	1.42
Nitzschia cf. fonticola Grun. in Van Heurck 1881	12	2.3	30.5	0.16	0.04	0.70
Nitzschia frustulum (Kütz.) Grun. in Cleve & Grun. 1880	65	18.8	71.9	16.52	0.35	620.29
Nitzschia graciliformis Lange-Bertalot & Simonsen 1978	25	13.0	4.7	0.30	0.05	1.96
Nitzschia gracilis Hantzsch 1860	17	13.1	2.2	0.35	0.09	1.35
Nitzschia cf. *gracilis*	8	2.3	48.2	1.15	0.14	9.38
Nitzschia hungarica Grun. 1862	12	4.6	5.0	6.43	0.28	145.21
Nitzschia aff. *intermedia* Hantzsch ex Cleve & Grun. 1880	7	4.1	2.7	0.35	0.03	3.91
Nitzschia liebetruthii Rabenh. 1864 fo. 1 PISCES	47	10.6	47.8	18.66	0.65	535.80
Nitzschia liebetruthii fo. 2 PISCES	19	6.7	28.6	3.33	0.54	20.32
Nitzschia obtusa W.Sm. 1853	10	5.2	3.3	21.43	3.54	129.72
Nitzschia palea (Kütz.) W.Sm. 1856	25	11.6	8.6	0.60	0.11	3.46
Nitzschia cf. *palea* var. *tenuirostris* sensu Lange-Bertalot 1976 nec. Grun. in Van Heurck 1881	20	6.3	18.5	0.75	0.10	5.75
Nitzschia paleacea (Grun. in Cleve & Grun.) Grun. in Van Heurck 1881	33	3.7	73.2	2.71	0.07	111.94
Nitzschia perminuta Lange-Bertalot 1980	18	7.6	43.3	38.02	12.76	113.24
Nitzschia cf. *perspicua* Chol. 1960 non Sovereign 1963	4	3.5	1.9	4.70	0.91	24.32
Nitzschia aff. *pusilla* Grun. 1862 emend. Lange-Bertalot 1976	5	1.8	6.8	11.04	0.58	209.41
Nitzschia radicula Hust. 1942	35	3.3	44.6	0.58	0.11	2.94
Nitzschia recta Hantzsch in Rabenh. 1861	14	9.1	2.8	0.16	0.03	0.95
Nitzschia sp. 1 PISCES	4	1.7	20.4	3.48	0.31	39.45
Nitzschia sp. 2 PISCES	4	2.2	44.9	17.06	13.74	21.18
Nitzschia sp. 3 PISCES (Cumming et al. 1995, Plate 37, Figs. 26 and 27)	7	2.3	17.9	0.85	0.18	4.06
Pinnularia appendiculata (Agardh) Cleve 1896	6	2.8	4.4	1.94	0.06	60.81
Pinnularia borealis var. *rectangularis* Carlson 1913	3	2.1	2.7	5.94	3.05	11.59
Pinnularia interrupta W.Sm. 1853	5	2.9	1.7	0.18	0.01	5.20
Pinnularia cf. *interrupta*	3	1.7	3.7	1.08	0.00	620.68
Pinnularia microstauron (Ehrenb.) Cleve 1891	19	10.7	2.6	0.86	0.02	48.64
Rhizosolenia cysts (converted to percent valves present)	4	2.7	2.5	0.05	0.02	0.14
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot 1980	10	3.0	8.7	1.32	0.03	63.10
Rhopalodia gibba (Ehrenb.) O.Müll. 1899	48	6.0	36.6	0.87	0.18	4.14
Rhopalodia gibberula (Ehrenb.) O.Müll. 1899	5	1.7	9.9	2.36	0.21	26.85
Stauroneis anceps fo. *gracilis* Rabenh. 1864	13	2.8	29.3	0.15	0.05	0.50
Stephanodiscus hantzschii Grun. in Cleve & Grun. 1880 (includes fo. *tenuis*)	19	5.0	37.3	0.26	0.02	3.31
Stephanodiscus hantzschii fo. 1 PISCES	9	4.1	4.9	0.23	0.03	1.96
Stephanodiscus medius Håkansson 1986	5	2.5	3.3	0.11	0.04	0.28
Stephanodiscus minutulus (Kütz.) Round 1981 (includes cf. *minutulus*)	77	20.4	83.2	0.21	0.02	2.03
Stephanodiscus niagarae Ehrenb. 1846	14	7.2	3.2	0.17	0.02	1.90
Stephanodiscus cf. *oregonicus* (Ehrenb.) Håkansson 1986	13	2.8	31.0	0.39	0.01	19.41
Stephanodiscus aff. *oregonicus*	8	2.5	14.4	0.27	0.02	3.46
Stephanodiscus parvus Stoermer & Håkansson 1984	72	22.9	68.8	0.15	0.02	1.09
Stephanodiscus sp. 1 PISCES	13	9.2	1.9	0.15	0.04	0.58
Surirella sp. 1 PISCES	11	2.5	31.1	4.40	0.67	29.04
Synedra delicatissima var. *angustissima* Grun. in Van Heurck 1881	12	2.7	9.3	0.34	0.06	1.91
Tabellaria flocculosa str. IIIp sensu Koppen 1975	25	9.3	8.0	0.18	0.00	10.09

Note: The salinity limits for each taxon were calculated as two abundance-weighted standard deviation units from the log salinity optimum. Upper salinity limit values that exceeded the highest measured lake water salinity in the data set ($620.29 \cdot g \cdot L^{-1}$) were replaced with this same value. The salinity optimum and tolerance ranges are shown as back transformations from the log late-summer salinity values. N2, effective number of occurrences (Hill 1973).

*Present only in the northern Great Plains data set.

© 1996 NRC Canada

Fig. 3. (*a*) The estimated salinity optima (solid squares) and tolerances (solid lines) of diatom taxa having salinity optima <0.37 g·L^{-1} and with an effective number of occurrences (N2) ≥ 3. The tolerances represent one abundance-weighted standard deviation unit. The taxa are arranged from high to low salinity optima.

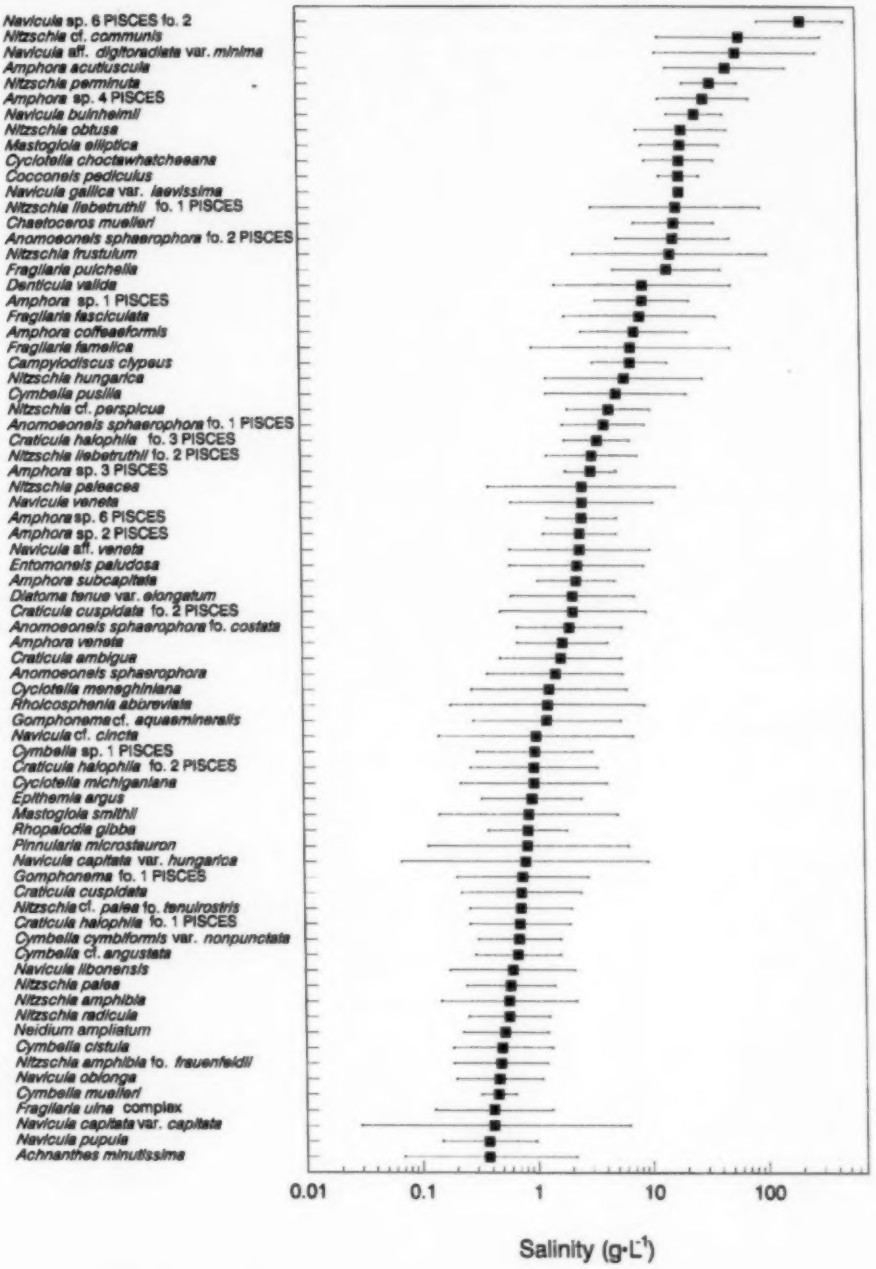


showed that, for diatom and pollen pH calibration data sets as well as other data sets, the lowest $RMSE_{boot}$ of prediction always occurs in WA regression and calibration prior to deletion of taxa on the basis of their effective number of occurrences (N2).

The initial improvement in the $WA_{(tol)}$ model when taxa with few occurrences were eliminated is not surprising, as tolerance estimates for relatively common taxa should be more reliable than those for less abundant taxa. This is supported by a ~40% improvement (decrease) in the $WA_{(tol)}$ s_1 component of the RMSE as N2 for a taxon's inclusion is increased from

© 1996 NRC Canada

Fig. 3. (*b*) The estimated salinity optima (solid squares) and tolerances (solid lines) of diatom taxa having salinity optima >0.37 g·L^{-1} and an effective number of occurrences (N2) ≥ 3. The tolerances represent one abundance-weighted standard deviation unit. The taxa are arranged from high to low salinity optima.

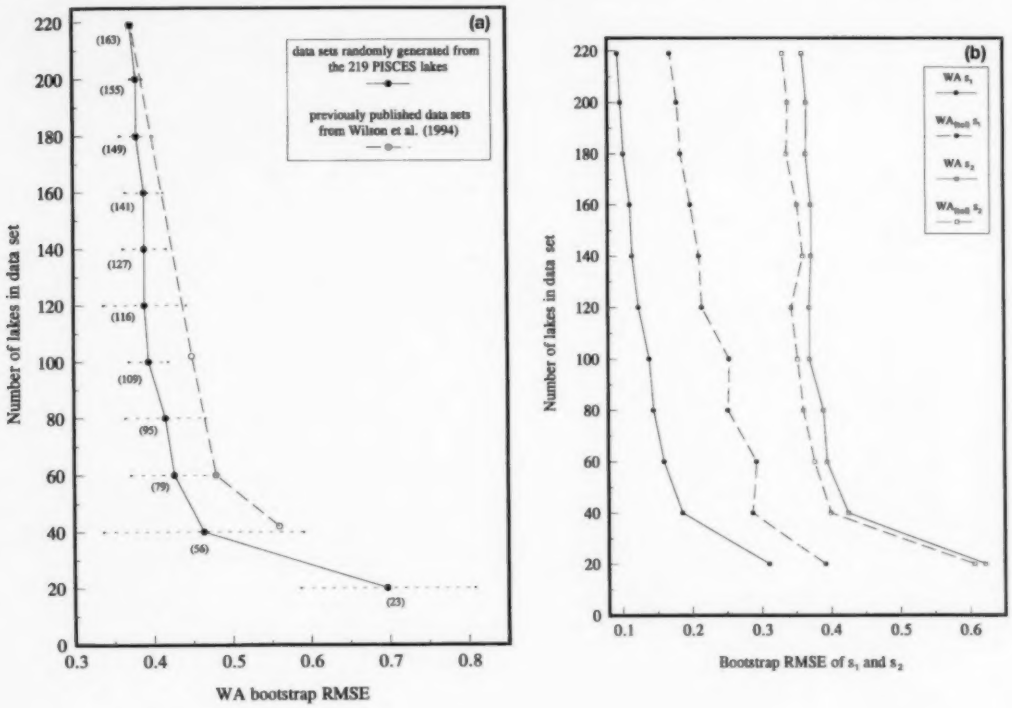


1 to 5 (Fig. 2*b*). However, both the WA and $WA_{(tol)}$ models show a steady increase in the overall $RMSE_{boot}$ as the criterion for inclusion of a taxon is increased past an N2 of 4 (Fig. 2*a*).

Although the lowest WA RMSE is observed at N2 = 1, there was relatively little difference in the RMSE when the deletion criterion was raised to an N2 of between 1 and 3. Notably, this is the zone where the rate of species loss is at a maximum (between N2 = 1 and N2 = 3, 55 taxa are lost from the model) (Fig. 2*a*) and indicates that the taxa that have been eliminated so far are rare enough to have had little impact on

© 1996 NRC Canada

Fig. 4. (*a*) A plot of the WA $RMSE_{boot}$ for salinity transfer functions generated randomly from the 219-lake data set. Data sets were generated at 20-lake intervals using a random number generator without replacement, performed five times for each interval. The mean $RMSE_{boot}$ from the five runs and the 95% confidence intervals are shown, along with the number of taxa present in the model (shown in parentheses). Also plotted are the $RMSE_{boot}$ for three previously published PISCES data sets (Wilson et al. 1994). In all cases the training set was comprised of taxa having relative abundances of at least 1% in three lakes, to compare with the earlier models developed by Wilson et al. (1994). (*b*) A plot of the mean RMSE of s_1 and s_2 for the randomly generated data sets depicted in *a*. Both the WA and $WA_{(tol)}$ salinity models are shown.



the predictive ability of the transfer function. This trend is more pronounced in the Birks (1994) study, where the RMSE of prediction in the diatom pH training set undergoes little change between an N2 of 1 and 16, suggesting that there is only a minor difference between models that use all available taxa and those that include taxa having anywhere from 2 to 16 effective occurrences.

The rapid deterioration in the WA and $WA_{(tol)}$ salinity models after N2 ~ 5 is related to the continued loss of diatom taxa from the model to below the critical number required for partial stability of the RMSE (~150) (Fig. 2*a*). Likewise, the distinct plateau in the RMSE observed between N2 = 8 and N2 = 15 in the diatom pH training set of Birks (1994) is most likely due to a minimal loss of common diatom taxa from the model during this interval.

In summary, both this study and the Birks (1994) study show that although the WA model with all taxa present does give the lowest $RMSE_{boot}$, there is often a region of stability where the RMSE will undergo little change when taxa are eliminated from the model. In the Birks (1994) study, this zone spans a much larger range in N2 (from 1 to 16) than in our study (N2 between 1 and 3), probably because fewer common taxa were being eliminated owing to greater redundancy (i.e., more taxa with high effective occurrences) in the data set.

Estimates of the salinity optima of the diatom taxa used in the WA and $WA_{(tol)}$ models show that approximately half of the 204 taxa have optima in the freshwater range (<0.5 $g \cdot L^{-1}$), 47 have optima in the subsaline range (0.5–3 $g \cdot L^{-1}$), 42 have optima in the saline range (3–50 $g \cdot L^{-1}$), and only 5 taxa have optima in the hypersaline range (>50 $g \cdot L^{-1}$) (Table 4). This largely reflects the decrease in diatom species diversity observed in lakes along the salinity gradient, a trend that has been confirmed from previous diatom studies of saline lakes (e.g., Gasse et al. 1983; Fritz et al. 1993; Wilson et al. 1994; Cumming et al. 1995). Additionally, the diatoms display a range of very narrow to broad tolerances across the entire log salinity gradient (Fig. 3). Only those taxa with relatively reliable tolerance estimates (N2 > 3) are shown.

Evaluating data-set size

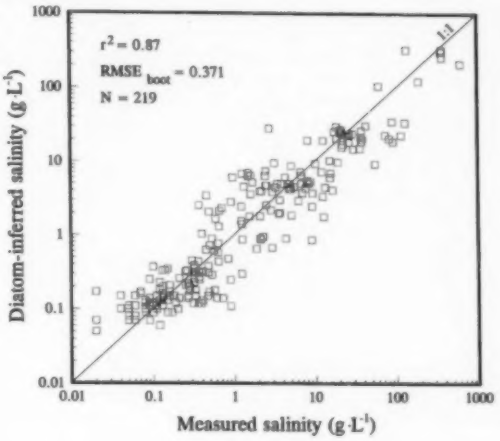
Along with a consideration of the number of taxa one should include in WA inference models, it is also important to evaluate the size of the data set. To assess the influence of data-set size (i.e., number of lakes in the training set) on the predictive ability of the WA and $WA_{(tol)}$ salinity inference models, we randomly generated, without replacement, training sets varying in size from 20 to 200 (in increments of 20, and five times for each size) from the 219-lake data set. The greatest improvement

© 1996 NRC Canada

in the $RMSE_{boot}$ was observed when the data set was enlarged from 20 to 60 lakes (Figs. 4*a* and 4*b*). After approximately 100 lakes, the RMSE remained relatively stable, with minor improvements between 160 and 219 lakes (Fig. 4*a*). This trend roughly parallels the plot of RMSEs of previously published transfer functions generated from subsets of the current data set (Wilson et al. 1994) (Fig. 4*a*). These data show that the RMSE of prediction continues to improve as more lakes and subsequently more taxa are included in the transfer function, yet past a certain point, in this case 100 lakes, the degree of improvement with the addition of lakes is relatively small. Therefore, to further improve the predictive ability (RMSE) of the present model to a noticeable degree, a very large number of lakes (100–200) would have to be added to the training set. Having said this, it is always preferable to sample as many lakes as possible to derive best estimates of species parameters to limnological variables. Estimates of taxa optima and tolerances are the driving force of our salinity model, and it is critical that these estimates should be based on as much information as possible. For example, 51% of the diatom taxa used in our current salinity model never reached relative abundances of >10%, and 16% of the estimated salinity optima were based on five occurrences or less (Table 4). As we increase the number of lakes in the data set, we continue to improve (lower) the s_1 component of the error in both the WA and $WA_{(tol)}$ models, signifying more consistent estimates of species optima and tolerances (Fig. 4*b*). Furthermore, the slopes of the s_1 curves did not stabilize, indicating that the optimum and tolerance estimates can be improved further. On the other hand, the s_2 errors, the largest component of the overall RMSE, were relatively stable after the training set was larger than 100–120 lakes (Fig. 4*b*), indicating that the noise produced by increasing the number of sites had stabilized. Initially we expected that as the number of sites was increased and subsequently the number of different diatom assemblages that could exist under similar environmental conditions, the s_2 component of the RMSE would also increase. In summary, once the training set has reached a size of 100–120 lakes, it is the decrease in the s_1 component of the error that is responsible for improvement in the overall RMSE when additional lakes are added to the training set.

Besides improving the estimates of species parameters, an additional benefit of a larger training set is that more taxa are included in the transfer functions, and the number of potential analogues for sediment core samples increases. However, even though we doubled the number of lakes used in the previous calibration (Wilson et al. 1994) to develop this transfer function, there are still a number of lake cores in which some of the reconstructed salinity values produced by the present transfer function are probably inadequate because certain abundant taxa in the sediment cores are not found in the surface calibration set. Therefore, we have to consider that these problems may be impossible to resolve with expansion of the data set simply because modern analogues may not exist. For example, high nutrient loading to many saline lakes, because of their location in areas of intense agriculture, makes it difficult to find analogues for highly saline, low-nutrient systems that presumably existed in the past. Alternatively, analogues may exist in geographical regions outside western Canada (e.g., Africa, Mexico, Australia), pointing to the potential advantage of larger, global data sets. This has been the aim of the CASPIA project (Juggins et al. 1994), which was established to compare saline-lake diatom floras from around the world and, ultimately, to merge regional data sets into a single data base of modern diatom and environmental data to generate interregional transfer functions for salinity reconstructions and to aid in the search for modern analogues of fossil assemblages.

Fig. 5. The relationship between measured salinity and diatom-inferred salinity after bootstrapping in the 219-lake training set. The measured salinity values represent late-summer salinity calculations of the major cations and anions, with the exception of the 11 northern Great Plains lakes that have midsummer salinity values.



Summary and conclusions

This study has emphasized the importance of including many taxa in inference models (transfer functions), both for the benefit of improving the WA RMSE of prediction and for providing more analogues of species assemblages encountered in lake sediment cores. As more lakes were included in the model, the number of taxa present in the transfer function increased and more consistent estimates of taxa optima and tolerances to salinity were generated. In this case, the $RMSE_{boot}$ of prediction was fairly stable after the training set reached 100 lakes; however, this may not be the case for other training sets that intend to reconstruct other variables (e.g., pH and total phosphorus). The addition of more lakes and subsequently more taxa to the training set in the future should continue to improve estimates of species parameters and we hope it will provide even better analogues for downcore assemblages. For example, the transfer function developed from this data set ($RMSE_{boot} = 0.37$; $r^2 = 0.87$) (Fig. 5) is an improvement over the earlier Wilson et al. (1994) model, which had a $RMSE_{boot}$ of 0.42.

Acknowledgements

The authors are grateful to Rick Nordin, Bob Grace, Norm Zirnhelt, Les MacDonald, and Vic Jensen from the British Columbia Ministry of the Environment for providing water chemistry analyses and logistical help in the field. A special thanks to Sheri Fritz for providing 11 diatom slides from her NGP data set and to John Glew for providing a map of the

© 1996 NRC Canada

study region. We thank H.J.B. Birks, John Kingston, and an anonymous reviewer for their comments on the manuscript. This research was made possible by a Graduate Dean's Travel Grant from Queen's University and funding from the Natural Sciences and Engineering Research Council.

References

Anderson, N.J. 1993. Natural versus anthropogenic change in lakes: the role of the sediment record. Trends Ecol. Evol. **8**: 356–361.

Birks, H.J.B. 1994. The importance of pollen and diatom taxonomic precision in quantitative palaeoenvironmental reconstructions. Rev. Palaeobot. Palynol. **83**: 107–117.

Birks, H.J.B., Line, J.M., Juggins, S., Stevenson, A.C., and ter Braak., C.J.F. 1990. Diatoms and pH reconstruction. Philos. Trans. R. Soc. London B Biol. Sci. **327**: 263–278.

Carvalho, L.R., Cox, E.J., Fritz, S.C., Juggins, S., Sims, P.A., Gasse, F., and Battarbee, R.W. 1996. Standardizing the taxonomy of saline lake *Cyclotella* spp. Diatom Res. **10**: 229–240.

Charles, D.F., and Smol, J.P. 1994. Long-term chemical changes in lakes: quantitative inferences using biotic remains in the sediment record. *In* Environmental chemistry of lakes and reservoirs. *Edited by* L. Baker. Adv. Chem. Ser. **237**: 3–31.

Cumming, B.F., Wilson, S.E., Hall, R.I., and Smol, J.P. 1995. Diatoms from British Columbia (Canada) lakes and their relationship to salinity, nutrients and other limnological variables. Bibl. Diatomol. No. 31.

Farley, A.L. 1979. Atlas of British Columbia: people, environment, and resource use. University of British Columbia Press, Vancouver, B.C.

Fritz, S.C., Juggins, S., Battarbee, R.W., and Engstrom, D.R. 1991. Reconstruction of past changes in salinity and climate using a diatom-based transfer function. Nature (London), **352**: 706–708.

Fritz, S.C., Juggins, S., and Battarbee, R.W. 1993. Diatom assemblages and ionic characterization of freshwater and saline lakes of the northern Great Plains, North America: a tool for reconstructing past salinity and climate fluctuations. Can. J. Fish. Aquat. Sci. **50**: 1844–1856.

Gasse, F., Talling, J.F., and Kilham, P. 1983. Diatom assemblages in East Africa: classification, distribution, and ecology. Rev. Hydrobiol. Trop. **16**: 3–34.

Gasse, F., Juggins, S., and Ben Khelifa, L. 1995. Diatom-based transfer functions for inferring past hydrochemical characteristics of African lakes. Palaeogeogr. Palaeoclimatol. Palaeoecol. **117**: 31–54.

Glew, J.R. 1991. Miniature gravity corer for recovering short sediment cores. J. Paleolimnol. **5**: 285–287.

Hall, R.I., and Smol, J.P. 1992. A weighted-averaging regression and calibration model for inferring total phosphorus concentration from diatoms in British Columbia (Canada) lakes. Freshwater Biol. **27**: 417–434.

Hill, M.O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology, **54**: 427–432.

Juggins, S., Battarbee, R.W., Fritz, S.C., and Gasse, F. 1994. The CASPIA project: diatoms, salt lakes, and environmental change. J. Paleolimnol. **12**: 191–196.

Line, J.M., ter Braak, C.J.F., and Birks, H.J.B. 1994. WACALIB version 3.3: a computer program to reconstruct environmental variables from fossil assemblages by weighted averaging and to derive sample-specific errors of prediction. J. Paleolimnol. **10**: 147–152.

Reyment, R.A. 1991. Multidimensional palaeobiology. Pergamon Press, Oxford.

Shi, G.R. 1993. Multivariate data analysis in palaeoecology and palaeobiogeography: a review. Palaeogeogr. Palaeoclimatol. Palaeoecol. **105**: 199–234.

Smol, J.P. 1995. Paleolimnological approaches to the evaluation and monitoring of ecosystem health: providing a history for environmental damage and recovery. *In* Evaluating and monitoring the health of large-scale ecosystems. *Edited by* D.J. Rapport, C.L. Gaudet, and P. Calow. Springer-Verlag, Berlin. pp. 301–318.

ter Braak, C.J.F., and Prentice, I.C. 1988. A theory of gradient analysis. Adv. Ecol Res. **18**: 271–313.

ter Braak, C.J.F., Juggins, S., Birks, H.J.B., and van der Voet, H. 1993. Weighted averaging partial least squares regression (WA-PLS): definition and comparison with other methods for species — environmental calibration. *In* Multivariate environmental statistics. *Edited by* G.P. Patil and C.R. Rao. Elsevier Science Publishers, Amsterdam. pp. 525–560.

Valentine, K.W.G., Sprout, P.N., Baker, T.E., and Lavkulich, L.M. 1978. The soil landscapes of British Columbia. British Columbia Ministry of the Environment, Victoria, B.C.

Wilson, S.E. 1996. Paleosalinity reconstructions using diatoms from lakes in western Canada. Ph. D. thesis, Dept. of Biology, Queen's University, Kingston, Ont.

Wilson, S.E., Cumming, B.F., and Smol, J.P. 1994. Diatom–salinity relationships in 111 lakes from the Interior Plateau of British Columbia, Canada: the development of diatom-based models for paleosalinity reconstructions. J. Paleolimnol. **12**: 197–221.

© 1996 NRC Canada

Main and interactive effects of invertebrate density, predation, and metals on a Rocky Mountain stream macroinvertebrate community

Peter M. Kiffney

Abstract: Using laboratory microcosms and a $2 \times 2 \times 2$ factorial design, I conducted an experiment to test the main and interactive effects of a mixture of metals (Cd, Cu, and Zn), invertebrate density, and predation on stream invertebrate community structure. Metal exposure reduced the abundance of one species: *Prostoia besametsa* (Plecoptera: Nemouridae). Presence of the predaceous stonefly *Hesperoperla pacifica* (Plecoptera: Perlidae) significantly affected invertebrate community structure, as total abundance ($p = 0.001$) and taxa richness ($p = 0.02$) were lower in predator-added treatments. Predation intensity on *Ephemerella infrequens* (Ephemeroptera: Ephemerellidae) was greater at high invertebrate density than at low density ($p = 0.01$). Metal exposure influenced the outcome of predation on *Hydropsyche* sp., as predation intensity was greater in streams dosed with metals than in controls ($p = 0.03$). Results from this experiment suggest that metals can influence the relative importance of biotic factors to alter stream invertebrate community structure.

Résumé : À l'aide de microcosmes de laboratoire et d'un plan factoriel $2 \times 2 \times 2$, j'ai mené une expérience visant à tester les effets principaux et interactifs d'un mélange de métaux (Cd, Cu et Zn), de la densité des invertébrés et de la prédation sur la structure de la communauté d'invertébrés d'un cours d'eau. L'exposition aux métaux a réduit l'abondance d'une espèce : *Prostoia besametsa* (Plecoptera : Nemouridae). La présence d'une perle prédatrice, *Hesperoperla pacifica* (Plecoptera : Perlidae), a affecté de façon significative la structure de la communauté des invertébrés, car l'abondance totale ($p = 0{,}001$) et la richesse des taxons ($p = 0{,}02$) étaient plus faibles après introduction du prédateur. L'intensité de la prédation sur *Ephemerella infrequens* (Ephemeroptera : Ephemerellidae) était plus forte à densité d'invertébrés élevée qu'à faible densité ($p = 0{,}01$). L'exposition aux métaux influait sur le résultat de la prédation exercée sur *Hydropsyche* sp., car l'intensité de prédation était plus forte dans les cours d'eau traités avec les métaux que dans les témoins ($p = 0{,}03$). Les résultats de cette expérience permettent de penser que les métaux peuvent influer sur l'importance relative des facteurs biotiques en modifiant la structure de la communauté d'invertébrés des cours d'eau.
[Traduit par la Rédaction]

Introduction

Environmental stress models (ESMs; Menge and Olson 1990) predict that the outcome of interactions among elements of a food web depends on the level of environmental stress. Two classes of ESMs, consumer stress models (CSMs) and prey stress models (PSMs), predict different outcomes depending on whether predators or prey are more affected by stress. Under high environmental stress, CSMs predict that predators will be more affected than their prey and predation rates should be reduced. As a result of lower predation rates, prey abundances will increase, leading to increased competition. Alternatively, PSMs predict that prey defenses are weakened more under high stress than predator activity. Thus, prey in high-stress environments are more susceptible to predation than those in low-stress environments (Menge and Olson 1990).

A review of field experiments from the marine intertidal zone provides empirical support for predictions of CSMs (Menge and Olson 1990). In severely wave-beaten sites, the abundance of sessile organisms was determined by physical stress and competition, whereas in more sheltered habitats, predation was more important. Studies from lotic ecosystems provide evidence, although equivocal, to support the notion that CSMs may predict effects of environmental stress on predator–prey interactions in stream invertebrate communities (Walde 1986; Peckarsky et al. 1990). For example, Walde (1986) showed that intensity of invertebrate predation was either eliminated or enhanced in streams at high levels of environmental stress (sedimentation).

Metal pollution is an important source of environmental stress to stream organisms in western North America. For example, over 3200 km of streams in Colorado receive metal-laden discharge from abandoned and active mines (Wilkinson 1992). The direct effects (e.g., changes in abundance or species richness) of metal exposure on stream macroinvertebrate communities in Rocky Mountain streams are fairly well known (Chadwick et al. 1986; Clements 1994; Kiffney and Clements 1994*a*, 1994*b*, 1996); however, very little information is available on whether metals interact with biotic processes to indirectly influence macroinvertebrate community structure. In the only study to date that tested the effects of metals on predator–prey interactions in stream invertebrate communities, Clements et al. (1989) observed that vulnerability of net-spinning caddisflies (Hydropsychidae) to predation was greater in copper-dosed microcosms (6 µg/L Cu) than in controls. In contrast to some studies (Walde 1986; Peckarsky et al. 1990), results from the Clements et al. (1989) experiment suggest that PSMs may predict the outcome of predator–prey

Received July 19, 1995. Accepted February 15, 1996.
J13006

P.M. Kiffney. EVS Consultants Ltd., 195 Pemberton Avenue, North Vancouver, BC V7P 2R4, Canada.

© 1996 NRC Canada

Table 1. Number of replicates per treatment combination for the $2 \times 2 \times 2$ factorial design.

	Low invertebrate density		High invertebrate density	
	No metals	Metals added	No metals	Metals added
No predators	2	2	2	2
Predators added	2	3	2	3

interactions in stream benthic communities when metals represent the environmental stress.

It has been observed in some western streams that invertebrate densities reach their highest level during fall (Leland et al. 1989; P.M. Kiffney, unpublished data). Biotic interactions (i.e., predation and (or) competition) may be the most intense at these high densities. As was mentioned previously, metal exposure represents a potentially important environmental stress to stream benthic organisms in the Rocky Mountains.

Using laboratory microcosms, I tested the main and interactive effects of invertebrate density, metals, and predation on a Rocky Mountain stream invertebrate community. Previous experiments have shown that large stonefly predators are more tolerant of metals than other invertebrate taxa (e.g., *Baetis* sp., Hydropsychidae) that are common in the diet of these predators (P.M. Kiffney, unpublished results). On the basis of these results, which confirm predictions of PSMs, I hypothesized that metal exposure would cause prey to be more susceptible to predation.

Materials and methods

Experimental system

General

To evaluate the effects of invertebrate density, metals, and predation on benthic invertebrate communities, artificial substrates were placed in the South Fork of the Poudre River (SFP), Colorado, a fourth-order stream in the Cache la Poudre catchment (Kiffney and Clements 1994*a*, 1994*b*). The artificial substrates consisted of $10 \times 10 \times 6$ cm plastic trays (0.01-m^2 surface area) filled with air-dried pebbles and small cobbles (2–6 cm diameter). Previous studies at SFP have demonstrated that macroinvertebrate assemblages colonizing trays were similar to those collected from surrounding natural substrates (Kiffney and Clements 1994*b*), except that overall macroinvertebrate densities were generally higher on trays and that densities of large predatory stoneflies (Plecoptera) were low.

Invertebrate density treatments

To manipulate invertebrate density, artificial substrates were colonized for either 13 days (36 trays) or 42 days (36 trays) in riffle sections of SFP during late summer and early fall 1993. Results from previous work had shown that after a 15-day colonization period, invertebrate abundance and species richness are approximately half that of artificial substrates colonized for 30 days (Clements 1991). Thus, I predicted that by varying substrate colonization time I would also vary invertebrate density. All trays were retrieved on the same day by placing a 100-μm mesh net directly downstream to prevent loss of organisms. Trays colonized for different time periods were kept separate and then randomly assigned to 18 plastic coolers (four trays per cooler) filled with stream water. Each cooler was aerated by an airstone connected to a 12-V air pump.

Four trays from each density treatment were randomly assigned to

Table 2. Water quality conditions of microcosms during the experimental period and in Little Beaver Creek (LBC) and the South Fork of the Poudre River (SFP) when trays and *Hesperoperla pacifica* were collected.

	Experimental streams	LBC	SFP
pH	7.51	7.5	7.7
Water temperature (°C)	11.1	6.0	8.0
Dissolved oxygen (mg/L)	7.8	11.0	10.1
Conductivity (mhos/s)	0.08	0.05	0.05
Hardness (mg/L)	30.1	10.0	20.0
Alkalinity (mg/L)	31.9	20.0	19.0
[Cd] (μg/L)	0.7±0.07*	nd	nd
[Cu] (μg/L)	6.0±0.4*	nd	nd
[Zn] (μg/L)	50.3±3.5*	nd	nd

Note: Samples were collected from experimental streams on days 0, 2, 4, and 7 ($n = 16$), whereas one sample was collected from each field site. Water quality conditions in control streams and metal-dosed streams were the same except for metal concentrations in treatment streams; therefore, values for control streams are omitted. nd, not detectable (detection limits of the analytical equipment were 0.1, 1.0, and 10.0 μg/L for Cd, Cu, and Zn, respectively).

*Statistically different from the control at $p < 0.05$.

oval, fiberglass experimental streams ($76 \times 46 \times 14$ cm; $n = 18$) in a greenhouse at Colorado State University, Fort Collins, Colorado. Microcosms receiving trays colonized for 13 days were designated low invertebrate density treatments, while those receiving trays colonized for 42 days were designated high invertebrate density treatments. Each flow-through stream received aerated, chilled, untreated water from nearby Horsetooth Reservoir at rate of 0.5 L/min. Turnover time in the 13-L streams was approximately 26 min at a water depth of 10 cm. Current was provided by a paddlewheel at an average velocity of 30 cm/s. Streams were exposed to a natural photoperiod.

Predation treatment

Hesperoperla pacifica (Plecoptera: Perlidae), a ubiquitous stonefly predator found in many streams in western North America (Stewart and Stark 1988), was collected from Little Beaver Creek, Colorado (LBC), a third-order stream also in the Cache la Poudre River catchment. During previous studies, densities of *Hesperoperla pacifica* ranging from 30 to 150/m^2 have been measured at LBC (P.M. Kiffney, unpublished results). *Hesperoperla pacifica* has been observed to feed on a variety of benthic invertebrates including *Baetis* sp., Chironomidae, and Trichoptera in experimental streams (P.M. Kiffney, unpublished results), and in field studies (Richardson and Gaufin 1971; Fuller and Stewart 1977; Johnson 1983). Previous experiments have also shown that this predator is tolerant of metal levels higher than that used in my experiment (P.M. Kiffney, unpublished results). Only stoneflies that were 15–20 mm long (tip of head to end of abdomen) were used. Stoneflies were transported to experimental streams in aerated coolers filled with natural stream water. Two densities of *H. pacifica* (based on area of artificial substrate) were added to experimental streams: one treatment received four stoneflies (100/m^2), while the other treatment received no stoneflies.

Treatment with metals

After a 48-h acclimation period, randomly selected microcosms were treated with a mixture of Cd, Cu, and Zn at target levels that were half the U.S. Environmental Protection Agency recommended chronic levels for water of less than 50 mg/L $CaCO_3$ (U.S. Environmental Protection Agency 1986) (i.e., 0.5, 6.0, and 55 μg/L of Cd, Cu, and Zn, respectively). These metal levels were also within the range of concentrations measured in metal-contaminated streams in Colorado

© 1996 NRC Canada

Table 3. *F* tests and statistical significance for overall ANOVA model, main effects, and two-way interactions for taxa richness, total abundance, and abundance of dominant taxa.

Variable	Model	Metals	Predation	Density	Density × predation	Density × metals	Predation × metals
Taxa richness	5.2***	0.9	6.9*	20.3***	0.1	3.8	0.01
Total abundance	9.0***	0.5	20.0***	31.0***	3.8	0.2	0.1
Rhithrogena hageni	0.6	0.1	1.3	1.5	0.01	0.3	0.4
Baetis tricaudatus	4.0*	1.1	14.5**	5.2*	1.3	0.4	0.3
Ephemerella infrequens	25.0***	1.0	11.2**	130.6***	9.3**	0.3	0.01
Pteronarcella badia	8.4**	0.04	16.0**	26.7***	3.0	0.4	4.1
Prostoia besametsa	8.4**	6.6*	8.5**	20.4***	3.3	0.1	15.7***
Hydropsyche sp.	9.7***	0.1	34.3***	9.1**	0.01	6.1*	6.1*

*$p < 0.05$.
**$p \leq 0.01$.
***$p \leq 0.001$.

(Clements 1994). Peristaltic pumps delivered a stock solution of $CdCl_2$, $CuSO_4$, and $ZnSO_4$ from separate, 20-L acid-washed carboys into each dosed stream at a rate of 5 mL/min. Because only 18 experimental streams were available, some treatments were replicated twice, whereas others were replicated in triplicate (Table 1). On the basis of previous work with this experimental system, it was hypothesized that treatments receiving both predators and metals would be the most variable; therefore, these treatments were replicated in triplicate. Thus, the full factorial experiment consisted of a planned, unbalanced $2 \times 2 \times 2$ treatment structure and a completely randomized design.

Data collection

After 7 days, the four trays in each stream were pooled and washed into a 355-μm mesh sieve, and retained organisms were preserved in 100% ethanol. Samples were sorted in the laboratory in white enamel pans and all organisms were identified to genus or species using available taxonomic keys and a reference collection.

Water samples were collected from LBC and SFP ($n = 1$) when trays were collected from the field, and on days 0, 2, 4, and 7 in experimental streams for water chemistry and metals analysis. Alkalinity and hardness were determined in the laboratory by titration (American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1989). Conductivity was measured with a conductivity meter (VWR Scientific, model 1054) and pH with a Chemcadet meter. Dissolved oxygen and water temperature were measured in field and laboratory streams with an O_2 meter (Yellow Springs Instrument Co., model 51B). All samples for metals analyses were acidified with reagent-grade HNO_3 to pH < 2. Cadmium and copper were analyzed on an IL 22 video dual channel atomic absorption spectrophotometer with a graphite furnace attachment, while zinc was measured on the same machine using a flame attachment. All metals were analyzed using standard methods (method No. 3111 A; American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1989). Metal levels in water are presented as total (suspended + dissolved) metals.

Statistical analysis

A three-way analysis of variance was used to test the main effects of invertebrate density, metals, and predation and all interactions (SAS Institute Inc. 1990). Treatment effects on taxa richness, total abundance, and abundance of dominant taxa (i.e., relative abundance > 0.1) were examined. Backward selection (criteria level < 0.05) was used to eliminate parameters that did not contribute significantly to the model. The only factor eliminated by this analysis was the three-way interaction. Estimates of means and standard errors were determined using SAS's LSMEANS (Milliken and Johnson 1992). If the *F* statistic was significant, *t* tests were used to compare means within the same effect. Because the experimental design was unbalanced, type III sum of squares was used in all analyses. Because inspection of residual plots indicated nonhomogeneity of variances, a square-root transformation was used to normalize variances.

Table 4. Taxa richness, total abundance, and abundance of dominant taxa (i.e., >0.1 relative abundance) per microcosm averaged over metal and predation treatments with artificial substates colonized in SFP for 13 versus 42 days.

	13 days	42 days
Taxa richness	14.6±0.6	18.0±0.6
Total abundance	317.1±12.4	414.4±12.4
Rhithrogena hageni	260.0±11.1	241.8±11.1
Baetis tricaudatus	18.2±1.1	21.7±1.1
Ephemerella infrequens	3.4±3.0	37.8±3.0
Pteronarcella badia	3.2±2.2	17.4±2.2
Prostoia besametsa	0.9±0.6	5.2±0.6
Hydropsyche sp.	5.5±0.5	8.4±0.5

Note: Values are given as the mean ± SE.

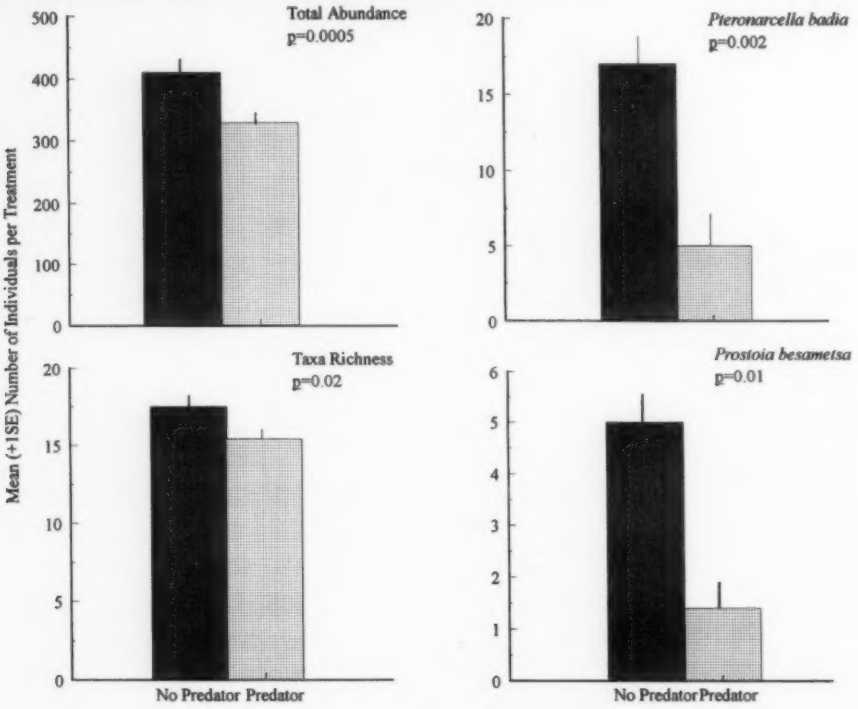
Results

Water quality in experimental and natural streams was similar except for lower water temperatures in the field and elevated metal concentrations in treated microcosms. Concentrations of Cd (0.7 ± 0.07; mean ± SE), Cu (6.5 ± 0.4), and Zn (50.3 ± 3.5) were significantly higher in metal-dosed microcosms than in controls (Table 2). Water concentrations of these metals (i.e., total metals) in controls and field sites were consistently below the detection limits of the analytical equipment. (Detection limits of Cd, Cu, and Zn were 0.1, 1.0, and 10.0 μg/L, respectively.)

Varying colonization time of artificial substrates influenced benthic invertebrate community structure in microcosms (Table 3). For example, abundances of dominant taxa, such as *Baetis tricaudatus* (Ephemeroptera: Baetidae), *Ephemerella infrequens* (Ephemeroptera: Ephemerellidae), *Pteronarcella badia* (Plecoptera: Pteronarcyidae), *Prostoia besametsa* (Plecoptera: Nemouridae), and *Hydropsyche* sp. (Trichoptera: Hydropsychidae) were significantly greater in microcosms with 42-day trays than microcosms with 13-day trays (Table 4). In contrast, the most abundant taxon, *Rhithrogena hageni*, was not affected by colonization time.

© 1996 NRC Canada

Fig. 1. Effects of the predator *Hesperoperla pacifica* on mean taxa richness, total abundance, and abundances of *Pteronarcella badia* and *Prostoia besametsa* per treatment averaged over density and metal treatments.



The addition of metals to microcosms had little effect on benthic community structure. *Prostoia besametsa* was the only species significantly reduced in metal-exposed microcosms (1.7 ± 0.84) compared with controls (4.8 ± 0.92; t test, $p = 0.03$).

The presence of *Hesperoperla pacifica* significantly reduced the abundance of most dominant taxa. Stonefly predation significantly reduced total density, taxa richness, and abundances of *Pteronarcella badia* and *Prostoia besametsa* (Fig. 1). Taxa common in the diet of *Hesperoperla pacifica* were also affected, as the abundance of *Baetis tricaudatus*, *E. infrequens*, and *Hydropsyche* sp. was significantly less in microcosms with predators versus those without predators (Fig. 2). Predator effects on the abundance of *E. infrequens* were observed only at high invertebrate density as indicated by the significant predator × density interaction (Fig. 3).

Metal exposure interacted with predation to affect the abundance of the caddisfly *Hydropsyche* sp. Specifically, the abundance of *Hydropsyche* sp. was reduced more in metal-dosed, predator-added microcosms than in predator-added microcosms without metals (predation × metals $p = 0.03$; Fig. 4). Although predation on *Pteronarcella badia* was 2 times greater in metal-dosed microcosms than in predator-added controls, the predator × metals interaction was not statistically significant ($p = 0.07$). *Prostoia besametsa* exhibited a different response than that of *Hydropsyche* sp. and *Pteronarcella badia*, as it was not affected by predators in metal-dosed microcosms, while abundance of this species was lower in predator-added controls (predation × metals, $p = 0.005$).

Discussion

Results of this experiment suggest that abiotic and biotic factors interact to influence benthic invertebrate community structure in stream microcosms. The caddisfly *Hydropsyche* sp. was more vulnerable to predation in microcosms exposed to low levels of metals, which was a response similar to that presented in Clements et al. (1989). These findings (i.e., this study and Clements et al. (1989)) suggest that prey stress models (Menge and Olson 1990) may predict the outcome of some invertebrate predator–prey interactions in metal-contaminated Rocky Mountain streams of Colorado.

Main effects

Laboratory and field experiments (Winner et al. 1980; Leland et al. 1986; Clements et al. 1988; Kiffney and Clements, 1994*a*, 1994*b*, 1996) and observational studies (Chadwick et al. 1986; Roline 1989; Clements 1994) have shown that metals can directly affect stream invertebrate community structure. Generally, mayflies and stoneflies are the most metal-sensitive groups, while caddisflies and chironomids are relatively tolerant. Because only one species (*Prostoia besametsa*) was significantly affected by metal treatment, my results suggest that these concentrations of Cd, Cu, and Zn (approximately one-half the U.S. Environmental Protection Agency (1986) chronic values) did not have a significant direct effect on stream invertebrates collected from SFP.

Although metal exposure was not an important factor influencing variation in invertebrate community structure, the

© 1996 NRC Canada

Fig. 2. Effects of *Hesperoperla pacifica* on mean abundance per treatment of three dominant taxa averaged over metal and density treatments.

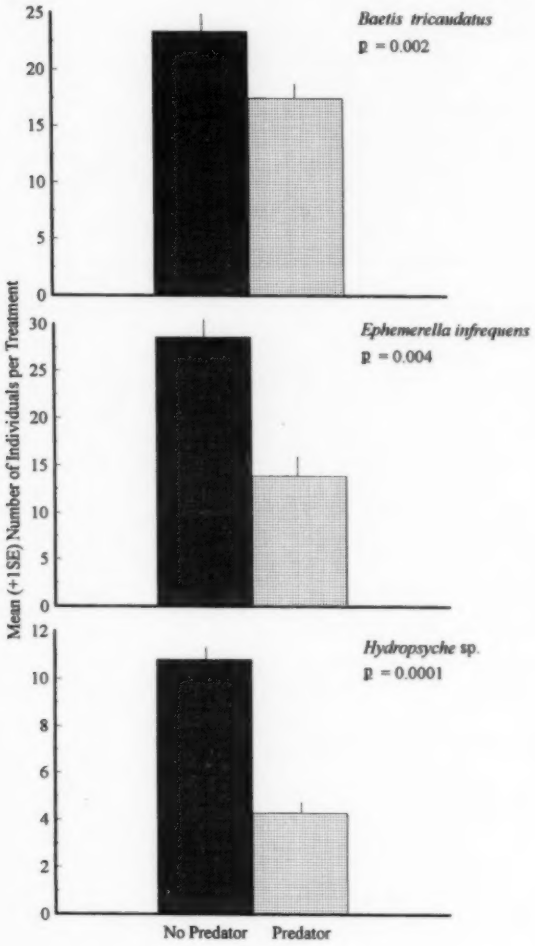
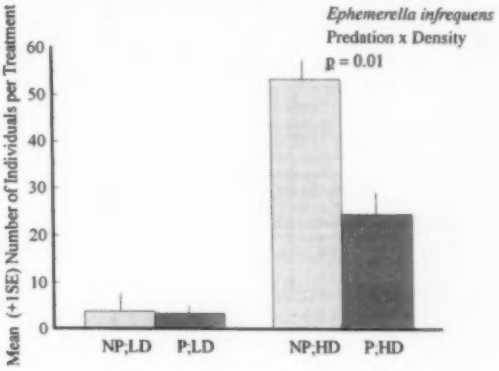


Fig. 3. Effects of *Hesperoperla pacifica* on mean abundance of *Ephemerella infrequens* in low- and high-density microcosms averaged over metal treatments. Treatments are NP, no predators; P, predators; LD, low invertebrate density; HD, high invertebrate density; NM, no metals; and M, metals.



presence of the stonefly predator, *Hesperoperla pacifica*, did significantly alter the benthic community. There has been considerable controversy over the importance of predation as an organizing force of stream benthic communities (Allan 1982; Reice and Edwards 1986; Peckarsky and Dodson 1980; Walde 1986). The stonefly *Megarcys signata* significantly depressed prey colonization in the East River, Colorado (Peckarsky and Dodson 1980). Walde (1986) showed that another invertebrate predator, *Kogotus modestus*, depressed densities of invertebrate prey, but this effect varied with abiotic factors (level of sedimentation). In my experiment, *Hesperoperla pacifica* significantly reduced the densities of most invertebrate taxa, especially those taxa (*Baetis* sp., *Hydropsyche* sp., and *E. infrequens*) that are known to be common prey items of *Hesperoperla pacifica* (Richardson and Gaufin 1971; Fuller and Stewart 1977; Johnson 1983). An unexpected result was a reduction in the abundance of *Pteronarcella badia*, a relatively large (but smaller than *Hesperoperla pacifica*), armored stonefly. This negative effect may be a result of direct predation; however, *Pteronarcella badia* was not found in the guts of *Hesperoperla pacifica* (P.M. Kiffney, unpublished results). Other researchers have shown that interference competition can occur between stonefly nymphs, reducing feeding rates of the smaller species (Peckarsky 1991). Thus, *Pteronarcella badia* may have been more susceptible to other factors, such as metals, as a result of biotic interactions with *Hesperoperla pacifica*.

The large predator impacts measured may have been an artifact of the experimental system. The semiclosed microcosms and the relatively high density of stoneflies used may have increased the probability of observing a significant predator impact (Cooper et al. 1990). Moreover, the small spatial and temporal scale of this experiment does not demonstrate that an invertebrate predator can significantly affect invertebrate community structure in natural streams. Nonetheless, these results do provide insights into processes, such as the interaction between biotic and abiotic factors, that may be occurring in stream invertebrate communities (Walde 1986).

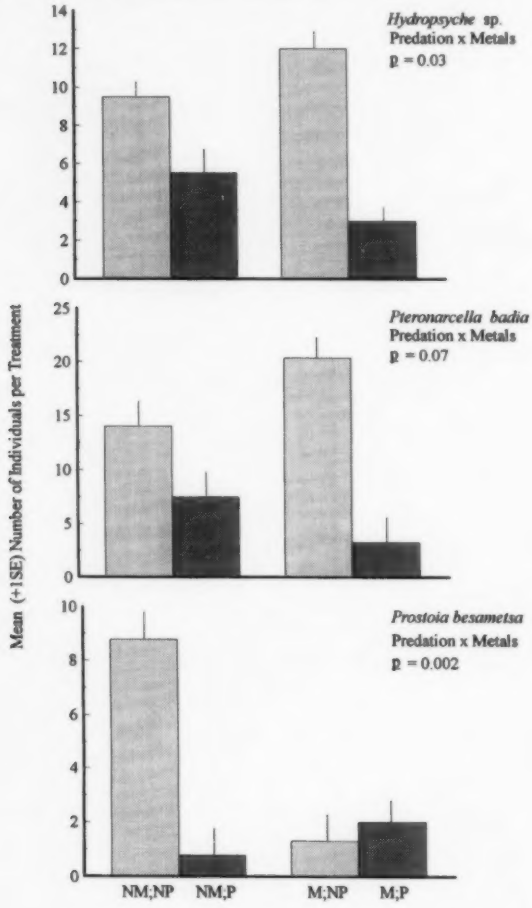
Interactive effects

Predation intensity on *E. infrequens* was greater at high invertebrate density, and similar results have been reported by other researchers. Significant reductions in abundance of various orthoclad chironomids by *Kogotus modestus* only occurred at high prey density (Walde and Davies 1984). Moreover, consumption rates of the predatory caddisfly *Plectrocnemia conspersa* were greater at high prey abundance, which may reflect a functional response of predators to prey density (Lancaster et al. 1990). A predator's effectiveness may be dependent on background prey density, and invertebrate predators may exert a greater effect at high prey density because prey refuges are less available.

Combined effects of metals and biotic processes were also complex and produced interesting patterns of community structure. Results of this study confirmed observations of Clements et al. (1989), who showed that vulnerability of hydropsychid caddisflies to a stonefly predator was greater in

© 1996 NRC Canada

Fig. 4. Effects of *Hesperoperla pacifica* on mean abundance of three dominant taxa in control and metal-dosed microcosms averaged over density treatments (see Fig. 3 for further details).



experimental streams dosed with low levels of Cu (6 µg/L) than in controls. Petersen and Petersen (1983) suggested that metals disrupt silk-spinning in Hydropsychidae, resulting in structural anomalies of the capture net. Clements et al. (1989) hypothesized that net-spinning caddisflies in metal-dosed streams are more vulnerable to predation because they spend more time outside retreats repairing and maintaining capture nets. Additional support for metal exposure affecting predation intensity was observed with the detritivorous stonefly *Pteronarcella badia,* although the predation × metals interaction term was not quite statistically significant ($p = 0.07$). These results imply that some benthic invertebrates, in particular hydropsychid caddisflies, may be at greater risk of predation in streams contaminated by metals.

Predation effects on *Prostoia besametsa* were in contrast to those observed for *Hydropsyche* sp. Because densities of *Prostoia besametsa* were much less in metal-dosed streams compared with controls, *Hesperoperla pacifica* may have not developed a search image for this taxon. Peckarsky and Penton (1989) speculated that stonefly attack probabilities may be frequency dependent; therefore, there should be a greater attack rate with the most frequently encountered prey.

Management implications

There is considerable variability in the sensitivity of benthic macroinvertebrates to metals (Winner et al. 1980; Leland et al. 1986; Clements et al. 1988; Kiffney and Clements 1994*a*, 1994*b*, 1996), and this variation in metal sensitivity may influence the importance of biotic interactions (Clements et al. 1989). The low concentrations (lower than chronic criteria of the U.S. Environmental Protection Agency) of metals dosed into stream microcosms did not have substantial direct effects on benthic community structure in this experiment; however, there were some unexpected indirect effects. If biotic interactions are more intense for some taxa in metal-contaminated streams, the structure of benthic invertebrate communities may be indirectly affected by metals. As single-species toxicity studies would not have been able to predict the indirect effects observed in my experiment, it is important to conduct more complex multispecies toxicity experiments using indigenous organisms, in conjunction with single-species tests, when assessing risk of contaminants on ecological systems (Clements et al. 1989). Results from such experiments may provide greater insights into how contaminants influence community structure, and what conceptual models may be most suitable in predicting effects of contaminants on biotic processes in aquatic environments. I suggest that PSMs may be a useful theoretical framework for predicting effects of metals on some predator–prey interactions and that "it is only through studying the interaction of biotic and abiotic factors that community structure can be fully understood" (Dunson and Travis 1990).

Acknowledgments

This research was supported by the U.S. Geological Survey (USGS), Department of Interior, under USGS award 14–08–0001-G2099, a Society of Environmental Toxicology and Chemistry pre-doctoral fellowship sponsored by the Procter and Gamble Company, the College of Natural Resources, and the Graduate Degree Program in Ecology at Colorado State University. W. Clements, K. Fausch, B. Kondratieff, C. Gowan, D. Byers, L. Hare, and one anyonomous reviewer provided valuable comments on earlier drafts of the manuscript. J. Benini, M. Pearson, and D. Hammerdorfer helped in the field and the laboratory. B. Kondratieff helped in identification of invertebrates, and P. Chapman provided statistical advice. S. Brinkman and P. Davies at the Colorado Division of Wildlife (CDOW) assisted in metals analysis. I also thank A. Ganek and the rest of the staff at the CDOW's Poudre River Fish Hatchery for the logistical assistance.

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard methods for the examination of wastewater. 17th ed. American Public Health Association, Washington, D.C.

Allan, J.D. 1982. The effects of reduction in trout density on the invertebrate community in a mountain stream. Ecology, **63**: 1444–1455.

Chadwick, J.W., Canton, S.P., and Dent, R.L. 1986. Recovery of benthic invertebrate communities in Silver Bow Creek, Montana,

© 1996 NRC Canada

following improved metal mine wastewater treatment. Water Air Soil Pollut. **28**: 427–438.

Clements, W.H. 1991. Characterization of stream benthic communities using substrate-filled trays: colonization, variability, and sampling selectivity. J. Freshwater Ecol. **6**: 209–221.

Clements, W.H. 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado. J. North Am. Benthol. Soc. **13**: 30–44.

Clements, W.H., Farris, J.L., Cherry, D.S., and Cairns, J. 1988. Structural alterations in aquatic insect communities to copper in laboratory streams. Environ. Toxicol. Chem. **7**: 715–722.

Clements, W.H., Cherry, D.S., and Cairns, J. 1989. The influence of copper exposure on predator–prey interactions in aquatic insect communities. Freshwater Biol. **21**: 483–488.

Cooper, S.D., Walde, S.J., and Peckarsky, B.L. 1990. Prey exchange rates and the impact of predators on prey populations in streams. Ecology, **71**: 1503–1514.

Dunson, W.A., and Travis, J. 1991. The role of abiotic factors in community organization. Am. Nat. **138**: 1067–1091.

Fuller, R.L., and Stewart, K.W. 1977. The food habits of stoneflies (Plecoptera) in the Upper Gunnison River, Colorado. Environ. Entomol. **6**: 293–302.

Johnson, J. 1983. Diel food habits of two species of setipalpian stoneflies (Plecoptera) in tributaries of the Clearwater River, Idaho. Freshwater Biol. **13**: 105–111.

Kiffney, P.M., and Clements, W.H. 1994*a*. Structural responses of benthic invertebrate communities from different stream orders to zinc. Environ. Toxicol. Chem. **13**: 389–395.

Kiffney, P.M., and Clements, W.H. 1994*b*. Effects of metals on a Rocky Mountain stream macroinvertebrate assemblage in experimental microcosms. J. North Am. Benthol. Soc. **13**: 511–524.

Kiffney, P.M., and Clements, W.H. 1996. Effects of metals on stream macroinvertebrate assemblages from different altitudes. Ecol. Appl. **6**: 472–481.

Lancaster, J., Hildrew, A.G., and Townsend, C.R. 1990. Stream flow and predation effects on the spatial dynamics of benthic invertebrates. Hydrobiologia, **203**: 177–190.

Leland, H.V., Fend, S.V., Dudley, T.L., and Carter, J.L. 1989. Effects of copper on species composition of benthic insects in a Sierra Nevada, California, stream. Freshwater Biol. **21**: 163–179.

Menge, B.A., and Olson, A.M. 1990. Role of scale and environmental factors in regulation of community structure. Trends Ecol. Evol. **5**: 52–57.

Milliken, G.A., and Johnson, D.E. 1992. Analysis of messy data: design of experiments. Van Nostrand Reinhold, New York.

Peckarsky, B.L. 1991. Mechanisms of intra- and interspecific interference between two larval stoneflies. Oecologia, **85**: 521–529.

Peckarsky, B.L., and Dodson, S.I. 1980. Do stonefly predators influence benthic distributions in streams? Ecology, **61**: 1275–1282.

Peckarsky, B.L., and Penton, M.A. 1989. Mechanisms of prey selection by stream-dwelling stoneflies. Ecology, **70**: 1203–1218.

Peckarsky, B.L., Horn, S.C., and Statzner, B. 1990. Stonefly predation along a hydraulic gradient: a field test of the harsh–benign hypothesis. Freshwater Biol. **24**: 181–191.

Petersen, L.B.M., and Petersen, R.C. 1983. Anomalies in hydropsychid capture nets from polluted streams. Freshwater Biol. **13**: 185–191.

Reice, S.R., and Edwards, R.L. 1986. The effect of vertebrate predation on lotic macroinvertebrate communities in Quebec, Canada. Can. J. Zool. **64**: 1930–1936.

Richardson, J.W., and Gaufin, A.R. 1971. Food habits of some western stonefly nymphs. Trans. Am. Entomol. Soc. (Phila.), **97**: 91–121.

Roline, R.A. 1988. The effects of heavy metal pollution of the upper Arkansas River on the distribution of aquatic macroinvertebrates. Hydrobiologia, **160**: 3–8.

SAS Institute Inc. 1990. SAS/STAT users' guide, release 6.04 edition. SAS Institute, Cary, N.C.

Stewart, K.W, and Stark, B.P. 1988. Nymphs of North American stonefly genera (Plecoptera). University of North Texas Press, Denton, Tex.

U.S. Environmental Protection Agency. 1986. Quality criteria for water 1986. EPA–440/5–86–001. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, D.C.

Walde, S.J. 1986. Effect of an abiotic disturbance on a lotic predator–prey interaction. Oecologia, **69**: 243–247.

Walde, S.J., and Davies, R.W. 1984. Invertebrate predation and lotic prey communities: evaluation of in situ enclosure/exclosure experiments. Ecology, **65**: 1206–1213.

Wilkinson, C.F. 1992. Crossing the next meridian: land, water, and the future of the west. Island Press, Washington, D.C.

Winner, R.W., Boesel, B.W., and Farrell, M.P. 1980. Insect community structure as an index of heavy metal pollution in lotic ecosystems. Can. J. Fish. Aquat. Sci. **37**: 647–655.

© 1996 NRC Canada

Improving pH and alkalinity estimates for regional-scale acidification models: incorporation of dissolved organic carbon

D.R. Marmorek, R.M. MacQueen, C.H.R. Wedeles, J. Korman, P.J. Blancher, and D.K. McNicol

Abstract: Dissolved organic carbon (DOC) can affect the accuracy of regional model projections of acid neutralizing capacity and pH. To improve predictive accuracy, we incorporated DOC into a modified titration curve and used a DOC-based correction factor to convert charge balance acid neutralizing capacity to an estimate of Gran alkalinity. Applying the new titration curve to 5612 lakes in Eastern Canada resulted in only a slight (0.08 unit) average improvement in the accuracy of pH predictions over a previous model without DOC. The DOC-based conversion of charge balance acid neutralizing capacity to Gran alkalinity had a greater impact on model projections: a median alkalinity decrease of 25.5 μequiv./L over 3226 lakes, lowering average pH projections for each of 25 subregions of Eastern Canada by 0.1–0.4 pH units. Empirical relationships calculated separately for each secondary watershed had slightly improved predictive power over regressions computed from the entire data set. These modifications produce quantifiable improvements in the accuracy of regional model estimates of pH and acid neutralizing capacity, and shift pH distributions towards more toxic levels.

Résumé : Le carbone organique dissous (COD) peut affecter l'exactitude des projections des modèles régionaux sur le potentiel de neutralisation des acides et le pH. Pour améliorer l'exactitude des prédictions, nous avons intégré le COD dans une courbe modifiée de titrage, et, à l'aide d'un facteur de correction basé sur le COD, nous avons converti le potentiel de neutralisation des acides pour l'équilibre ionique en une estimation de l'alcalinité Gran. L'application de la nouvelle courbe de titrage à 5612 lacs de l'est du Canada n'a apporté qu'une légère (0,08 unité) amélioration moyenne de l'exactitude des prévisions du pH par rapport à un modèle antérieur n'utilisant pas le COD. La conversion basée sur le COD a eu un impact plus grand sur les projections des modèles : baisse médiane de l'alcalinité de 25,5 μequiv./L pour 3226 lacs, ce qui diminue les projections du pH moyen pour chacune des 25 sous-régions de l'est du Canada de 0,1 à 0,4 unité de pH. Les relations empiriques calculées séparément pour chaque bassin secondaire avaient légèrement amélioré la puissance de prédiction par rapport aux régressions calculées avec l'ensemble de la série de données. Ces modifications apportent des améliorations quantifiables à l'exactitude des estimations des modèles régionaux du pH et du potentiel de neutralisation des acides, et font passer les distributions du pH à des niveaux de plus grande toxicité.
[Traduit par la Rédaction]

Introduction

Regional-scale models of the effects of acid deposition have played a prominent role in both formulating policy and focusing research and monitoring activities for fish, wildlife, and invertebrates (Kamari et al. 1989; Thornton et al. 1990; Minns et al. 1990; Jones et al. 1990; Blancher et al. 1992; McNicol et al. 1995). Most of the simpler steady-state models have a common three-part structure: (*i*) predict acid neutralizing capacity (ANC), (*ii*) estimate pH from the predicted ANC, and (*iii*) estimate biological effects from pH (and sometimes calcium and aluminum) (Baker et al. 1990; Thornton et al. 1990). Though some recently developed models predict impacts on fish directly from ANC (Bulger et al. 1993; Cosby et al. 1994), there is considerable evidence that pH is the most critical environmental variable causing damage to aquatic biota (Baker et al. 1990). Thus, accurate predictions of pH on regional scales are critical to defensible estimates of biological change. There are, however, substantial variations in the definitions of ANC among models (Sullivan et al. 1989; Thornton et al. 1990; Hemond 1990) and in the degree to which dissolved organic carbon (DOC) is considered.

In this paper, we explore some simple approaches to improving regional model ANC and pH estimates, particularly for brown waters, by incorporating DOC. The first set of analyses examines titration curves, specifically the prediction of pH from Gran alkalinity (Gran Alk, the equivalent sum of titratable bases to the Gran procedure end point) and DOC. We compare observed pH levels with pH predictions from three different titration curves: the ESSA–DFO curve (Marmorek et al. 1990), Small and Sutton's (1986) curve, and a modification of Small and Sutton's curve that explicitly accounts for

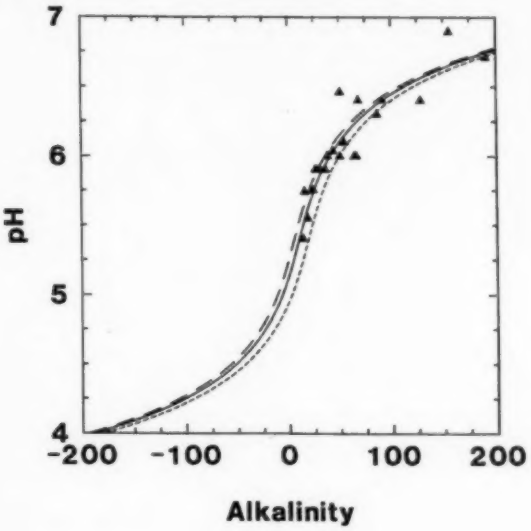
Received April 20, 1995. Accepted February 15, 1996.
J12881

D.R. Marmorek, R.M. MacQueen, and J. Korman. ESSA Technologies Ltd., Suite 300, 1765 West 8th Avenue, Vancouver, BC V6J 5C6, Canada.
C.H.R. Wedeles. ESSA Technologies Ltd., Suite 308, 9555 Yonge Street, Richmond Hill, ON L4C 9M5, Canada.
P.J. Blancher. Canadian Wildlife Service, National Wildlife Research Centre, 100 Gamelin Boulevard, Hull, QC K1A 0H3, Canada.
D.K. McNicol.[1] Canadian Wildlife Service, Ontario Region, 49 Camelot Drive, Nepean, ON K1A 0H3, Canada.

[1] Author to whom all correspondence should be addressed.

© 1996 NRC Canada

Fig. 1. Modified Small and Sutton (1986) curve fit to entire data set, calculated on the basis of the median DOC from the data set, 4.7 mg/L (solid line). The line with long dashes is the same curve plotted for the first decile from the data set (2.4 mg/L). The line with short dashes is the same curve plotted for the ninth decile (9.0 mg/L). Triangles represent median observed Gran Alk and median observed pH for all secondary watersheds studied except 4J (off scale).



each lake's DOC. Our second set of analyses examines the effect of incorporation of DOC on estimates of ANC. We derive an empirical correction factor to be subtracted from model predictions of charge balance ANC (CBANC; base cations minus strong acid anions) to produce a closer estimate of Gran Alk, permitting model projections to be compared directly with measured Gran Alk. Both of these approaches generate estimates of charge density (the microequivalents of organic anion per milligram of DOC required for charge balance). We then examine the net result of these corrections on regional model predictions.

As pointed out by both Sullivan et al. (1989) and Hemond (1990), CBANC and Gran Alk are only equivalent when DOC = 0; otherwise CBANC exceeds Gran Alk. Hemond (1990) showed that theoretically this difference should be 4.6 μequiv. of ANC/mg DOC. Therefore, model projections of CBANC and measurements of Gran Alk are not directly comparable for water with DOC > 0 (i.e., most surface waters). When all seven major base cations and acid anions are measured, the predicted and observed CBANC can be directly compared. However, the cumulative effect of analytical errors in the seven major ions, or missing measurements, can limit the utility of such comparisons for model validation. Comparisons of predicted CBANC and observed Gran Alk offer a second means of model testing.

Methods

DOC can affect acidification model projections in at least two ways. First, DOC affects the calculation of pH from Gran Alk or CBANC. Many regional-scale models perform this calculation by empirically fitting theoretically based equations to regional survey data (Thompson 1982; Wright and Henriksen 1983; Schnoor et al. 1984; Small and Sutton 1986; Marmorek et al. 1990). A water sample with organic anions will have a lower pH than another sample with identical Gran Alk, but no organic anions (Hemond 1990). Thus, DOC should be incorporated into the calculation of pH, ideally with complete chemistry measurements (e.g., Clair et al. 1992), though these are often not available on a regional scale. Second, dissociation of DOC affects Gran Alk by increasing the quantity of organic anions, while at the same time adding H^+. The overall effect of DOC on Gran Alk is dependent on the number and type of organic acid functional groups present and the surface water pH (Perdue 1978).

Dissolved organic carbon and pH–alkalinity relationships

Small and Sutton titration curve

Small and Sutton (1986) developed a semi-empirical relationship between Gran Alk and pH that may be fit to regional data sets and used for predicting pH from model alkalinity projections. Their titration curve uses nonlinear parameter estimation to estimate a, c, and d in the following equation:

$$(1) \quad \mathrm{pH} = a + \frac{1}{\ln 10} \times \operatorname{arcsinh} \frac{(\mathrm{Alk} - d)}{c}$$

where Alk is Gran alkalinity in microequivalents per litre, arcsinh is the inverse hyperbolic sine function, d is related to the data set's average effective organic anion concentration in microequivalents per litre, and a and c are functions of P_{CO_2}, carbonic acid dissociation constants, and CO_2 saturation.

When estimating the parameter values for Small and Sutton's (1986) titration curve, only pH and Gran Alk are required. Small and Sutton point out that various parameters can absorb variation in pH, so that d may not accurately reflect organic anion concentrations. To explicitly incorporate DOC, we modified eq. 1 as follows:

$$(2) \quad \mathrm{pH} = a + \frac{1}{\ln 10} \times \operatorname{arcsinh} \frac{(\mathrm{Alk} - b \times \mathrm{DOC})}{c}$$

where DOC is the measured concentration of DOC in milligrams per litre and b is the average microequivalents of organic anions per milligram of DOC (i.e., the charge density). In this relationship, DOC represents an actual concentration used in conjunction with pH and alkalinity to fit the parameters a, b, and c. We attempted this modification because we expected less regional variation in charge density than in organic anion concentrations and therefore a better fit.

Test data set

The region of concern was defined as the area of Canada east of the Ontario–Manitoba border and south of latitude 52°N, the same area considered by Jones et al. (1990) in their application of the ESSA–DFO regional acidification model. For consistency with that and other work, we chose 26 secondary watersheds (one level below primary watersheds such as the St. Lawrence River) as the spatial units used for all analyses (see Jones et al. 1990, Fig. 1). The data set consists of 5612 records in total, from three separate sources: (*i*) the 1990 Canadian Long Range Transport of Air Pollutants and Acid Deposition Assessment report (Federal–Provincial Research and Monitoring Coordinating Committee 1990), excluding data from secondary watersheds wholly within Ontario (hereafter referred to as the LRTAP data set); (*ii*) an Ontario Ministry of Environment and Energy data set (MOE; Neary et al. 1990); and (*iii*) the Quebec Lake Survey data set (Dupont 1993) (hereafter referred to as the QLS data set). We excluded from our analyses secondary watersheds for which the combined data set (Table 1) contained fewer than 40 records. We did not attempt to exclude lakes that may have been included in more than one of the three source data sets, because we are only using the data to examine chemical relationships, not to make regional predictions

© 1996 NRC Canada

Table 1. Origin of lake data and results of calculating pH on the basis of various fits to the alkalinity and DOC data, by secondary watershed, for the entire data set (ALL) and for a 1000-point random subsample of the entire data set.

					ESSA–DFO model[d]		Unmodified Small and Sutton[e] (mean abs. resid.)	Modified Small and Sutton[f] (mean abs. resid.)	Regional modified Small and Sutton[g]	
Watershed	LRTAP[a]	MOE[b]	QLS[c]	*n*	Mean abs. resid.	Mean resid.			Mean abs. resid.	Mean resid.
1A	92	0.0	0.0	92	0.193	0.150	0.124	0.100	0.113	−0.027
1D	89	0.0	0.0	89	0.282	0.233	0.207	0.178	0.184	−0.008
1E	207	0.0	0.0	207	0.317	0.288	0.214	0.175	0.176	0.019
1F	69	0.0	0.0	69	0.336	0.232	0.264	0.261	0.284	0.028
2B	0.0	342	0.0	342	0.177	−0.024	0.175	0.167	0.230	−0.168
2C	42	94	0.0	136	0.171	−0.093	0.149	0.145	0.262	−0.193
2D	198	171	0.0	369	0.163	−0.027	0.152	0.161	0.243	−0.094
2E	0.0	546	0.0	546	0.250	0.175	0.193	0.184	0.192	−0.024
2H	0.0	142	0.0	142	0.238	0.206	0.160	0.152	0.155	0.068
2J	308	181	143	632	0.309	0.206	0.250	0.227	0.243	0.025
2K	298	457	0.0	755	0.194	0.135	0.163	0.145	0.157	−0.048
2L	167	2	174	343	0.382	0.367	0.207	0.180	0.214	0.143
2N	223	0.0	198	421	0.379	0.367	0.182	0.150	0.188	0.131
2O	74	0.0	22	96	0.324	0.131	0.235	0.231	0.244	0.008
2P	88	0.0	46	134	0.310	0.299	0.146	0.141	0.172	0.113
2R	224	0.0	207	431	0.311	0.260	0.228	0.213	0.229	0.050
2S	17	0.0	61	78	0.278	0.248	0.214	0.170	0.183	0.002
2T	25	0.0	55	80	0.276	0.163	0.229	0.199	0.211	−0.106
2U	18	0.0	26	44	0.357	0.273	0.252	0.202	0.209	−0.028
2V	15	0.0	28	43	0.251	0.160	0.186	0.129	0.207	−0.103
2W	13	0.0	27	40	0.320	0.187	0.178	0.152	0.201	−0.083
2Y	93	0.0	0.0	93	0.180	0.096	0.169	0.149	0.226	−0.163
3A	14	0.0	162	176	0.492	0.489	0.187	0.168	0.268	0.242
3B	0.0	0.0	68	68	0.353	0.326	0.178	0.179	0.241	0.171
4G	0.0	49	0.0	49	0.368	0.364	0.257	0.227	0.267	0.200
4J	0.0	137	0.0	137	0.380	−0.292	0.241	0.241	0.274	−0.179
Total	2274	2121	1217							
Average					0.292	0.189	0.196	0.178	0.198	−0.001
ALL				5612	0.309	0.227				

Note: Mean residual (mean resid., $pH_{calculated} - pH_{observed}$) shows overall bias (= 0 if no consistent bias); mean absolute residual (mean abs. resid., $|pH_{calculated} - pH_{observed}|$) shows level of fit (the lower the better).

[a]Long Range Transport of Air Pollutants and Acid Deposition Assessment data.

[b]Ontario Ministry of Environment and Energy data.

[c]Quebec Lake Survey data.

[d]Marmorek et al. (1990) curve for the entire data set.

[e]Fit to data in each secondary watershed.

[f]Fit to data in each secondary watershed.

[g]Fit to a 1000-point random subsample of the entire data set.

of acidification. In any case, the overlap is small. For secondary watershed 2L, with roughly equal numbers of LRTAP and QLS records (see Table 1), fewer than 20 pairs out of a total of 343 records (less than 6%) originated from sites closer together than 20 km.

The lakes are characterized by a wide range of chemical conditions (Figs. 1 and 2, Table 2). Secondary watershed median pH varies from lower than 5.4 (secondary watershed 1E) to almost 8.0 units (secondary watershed 4J: not shown in Fig. 1), and median Gran Alk varies from 14 to 2004 μequiv./L. More than half of the DOC values lie in the range from 2 to 10 mg/L (Fig. 2).

Titration curve estimation

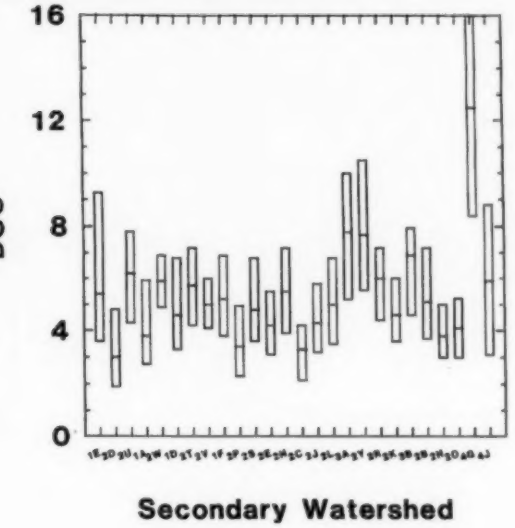
We used nonlinear least squares parameter estimation (Wilkinson 1990) to estimate parameter values for the modified and the unmodified Small and Sutton (1986) titration curves. This procedure minimizes squared deviations of the dependent variable (pH) data values from values estimated by the function at the same independent variable (Alk and DOC) data points. The parameter estimates were completed for all secondary watersheds separately, and for a 1000-point random subsample of the combined data set (the regional fit). Repeated sampling from the MOE data set by Korman et al. (1991) verified that choosing such a random subsample of the data does not significantly affect parameter estimates. We assessed the bias of fitted curves by computing the average value of the residual ($pH_{calculated} - pH_{observed}$). Since curves could have low overall bias but still fit the data poorly, we also computed the average absolute value of residuals as a measure of goodness of fit.

Incorporating DOC into ANC projections

To estimate ANC in pre-industrial times, the ESSA–DFO model (Marmorek et al. 1990) uses a simplified estimate of original CBANC

© 1996 NRC Canada

Fig. 2. Median DOC and middle quartiles (25–75%) for all secondary watersheds. The bottom of each box represents the first quartile, and the top represents the third.



([CBANC*]$_0$), which ignores constituents that are insignificant in the absence of acidic deposition, that is

(3) estimated $[CBANC^-]_0 = [C_B^{n+}]_0 - [Cl^-]_0 - [SO_4^{2-}]_0$

How good or bad an estimate of pre-industrial [Gran Alk]$_0$ does eq. 3 provide? Organically acidified (high DOC) lakes should have had a lower [Gran Alk]$_0$ than lakes with low DOC, but eq. 3 does not explicitly take this into account. We performed two analyses to address this issue. First, we defined an estimated current ANC:

(4) estimated $[CBANC^-]_t = [C_B^{n+}]_t - [Cl^-]_t - [SO_4^{2-}]_t$

analogous to the approximation in eq. 3, and compared ($[CBANC^*]_{t,estimated}$ – [Gran Alk]$_{t,observed}$) with DOC concentrations.

On the basis of these analyses, we concluded that the ESSA–DFO model partial CBANC generally overestimates [Gran Alk]$_0$ and that the extent of this overestimate increases in lakes with high DOC (Fig. 3). This leads to an overestimate of pH. To address this problem, we took the pragmatic approach of estimating a correction factor from a linear regression of alkalinity residuals ($[CBANC]_{t,estimated}$ – [Gran Alk]$_{t,observed}$) versus DOC. We calculated correction factors both on a secondary watershed by secondary watershed basis, and also on a regional basis (for the data set as a whole). The correction factor may be thought of as a device for converting CBANC to Gran Alk, which is the commonly measured alkalinity required by Small and Sutton's (1986) titration curve.

Test data set

The data set used for this analysis is a subset of the data set used in the titration curve analyses (3226 of the 5612 lakes); lakes without sufficient chemical data to calculate CBANC* were excluded. In almost one third of the secondary watersheds studied this meant a reduction of the sample size by more than 50% and in one case (4J) no complete records were available. Records with measured Gran Alk greater than 200 μequiv./L were excluded from the data set because the relative effect of organic anions on CBANC* in these systems is small. Including these records would increase the error of the correction factor.

Results and discussion

Comparison of ESSA–DFO and Small and Sutton (1986) titration curves

Parameter values for the unmodified Small and Sutton (1986) titration curve regional fit (eq. 1; fit based on a 1000-point subsample of the full data set) were $a = 5.31 \pm 0.03$ (mean ± SE); $c = 14.7 \pm 0.8$; $d = 5.6 \pm 1.3$. The d parameter provides estimates of organic acid anions in microequivalents per litre. Parameter values for a similar fit of the modified Small and Sutton (1986) curve (eq. 2) were $a = 5.39 \pm 0.02$; $b = 2.1 \pm 0.2$; $c = 16.1 \pm 0.7$. Here b represents the average charge density, or number of microequivalents of organic anions per milligram DOC. This estimate of charge density is lower than that of most other studies (Table 3). We believe this is because some of the effect of DOC on pH is absorbed by the a and c parameters, rather than by b. Small and Sutton found that their model (eq. 1) also underestimated the amount of organic acids.

Variation in DOC changes pH by less than 0.2 units when Gran Alk is >50 μequiv./L or <25 μequiv./L (Fig. 1). Where DOC has the greatest effect, for alkalinity 0, the estimated pH differs by only 0.3 units between the first and ninth deciles of DOC for the combined data set (Fig. 1). The data points in Fig. 1, median pH and alkalinity for each secondary watershed, compare well with the regional titration curve.

Table 1 compares the fits of the ESSA–DFO titration curve with both the unmodified and the modified Small and Sutton (1986) titration curves. The ESSA–DFO curve overestimates pH, for all but four secondary watersheds, to a maximum of almost 0.5 units (secondary watershed 3A). The overestimate for the data set as a whole (mean residual) is about 0.2 units. The pH underestimate for secondary watershed 4J (median pH = 7.95) is due to a pH cap of 7.66 in the ESSA–DFO curve. When fit to each secondary watershed, the modified Small and Sutton (1986) curve fits the data slightly better than the unmodified curve (0.02 pH units overall, lower residuals in all but two watersheds). The regional modified Small and Sutton (1986) curve fits the data better than does the ESSA–DFO curve and has less bias. Both watershed-specific Small and Sutton curves show a slightly better overall fit (average absolute residual, Table 1) and slightly lower bias (not shown in Table 1) than the regional modified Small and Sutton curve. This may be partly due to regional variation in the nature of DOC. As in Wilkinson et al. (1992), we found that charge density decreased at higher DOC levels, so that watershed-specific curves can better track their particular DOC characteristics. The decrease in charge density at high DOC levels suggests that a nonlinear term for DOC in eq. 1 may provide improved fits to the data for regional curves, but we did not explore this further.

Comparison of corrected charge-balance alkalinity and Gran alkalinity

The simplified estimate of CBANC (CBANC*, eq. 4) overestimates Gran Alk to a greater degree in lakes with higher DOC (Fig. 3). The underestimate of Gran Alk at low DOC levels (Fig. 3) is most likely due to not including uncomplexed forms of aluminum in eq. 4. On the basis of patterns of residuals like this, we decided to estimate a correction factor from the residuals versus DOC regressions, both on an individual secondary

© 1996 NRC Canada

Table 2. Application of a correction factor (CF) to charge-balance alkalinity.

Watershed	n	Slope	Intercept	p	Mean DOC	Mean Gran Alk	Reg. CF DOC (WS)	Fit: No CF avg. abs. resid.	Fit: Reg. CF avg. abs. resid.	Fit: WS CF avg. abs. resid.	Bias: No CF avg. resid.	Bias: Reg. CF avg. resid.	Bias: WS CF avg. resid.
1A	80	2.5	0.9	<0.001	4.4	26.3	22	15.0	12.5	8.8	12.0	−9.6	0.0
1D	84	3.0	2.0	<0.05	5.3	38.4	26	30.2	23.8	23.2	18.3	−7.5	0.6
1E	207	2.0	2.0	<0.001	6.9	19.9	35	25.2	26.1	20.4	15.5	−19.1	−0.3
1F	64	−1.0	27.0		5.8	43.4	29	23.8	22.8	18.3	20.4	−8.2	−0.8
2B	124	5.8	−10.0	<0.001	5.1	65.1	25	18.9	8.5	7.0	18.2	−5.6	−0.1
2C	51	5.1	−20.0		3.1	40.5	15	39.9	39.8	39.0	−5.1	−19.0	−0.4
2D	212	8.5	−31.0	<0.001	3.4	21.0	16	26.4	25.1	18.6	−6.4	−19.6	0.3
2E	202	6.2	−5.1	<0.001	4.6	49.0	22	27.1	12.9	12.8	23.9	1.1	0.1
2H	43	0.8	24.0		3.7	67.2	18	28.1	17.9	14.6	26.7	11.0	0.0
2J	307	7.0	−9.0	<0.001	4.9	54.7	24	33.2	16.4	15.9	27.5	2.0	0.2
2K	122	6.3	−7.0	<0.001	5.2	78.1	26	35.3	14.3	14.1	31.9	1.3	0.1
2L	285	6.3	−3.0	<0.001	5.7	61.7	28	34.5	13.3	12.6	32.4	4.4	−0.3
2N	407	4.8	13.2	<0.001	5.8	60.2	29	41.7	16.1	11.0	41.5	12.6	0.3
2O	43	10.0	−7.0	<0.01	3.9	72.6	19	36.6	23.9	25.2	32.2	13.5	0.1
2P	114	3.0	−0.1		3.8	50.6	18	27.0	22.6	23.2	11.1	−6.7	0.1
2R	342	6.4	4.8	<0.001	6.0	80.5	30	44.1	18.1	13.8	43.0	13.2	−0.2
2S	74	5.6	1.1	<0.001	5.5	48.5	27	32.7	11.0	10.4	31.7	4.5	−0.3
2T	73	4.6	4.1	<0.001	5.8	37.7	29	32.1	10.7	10.5	31.3	1.9	−0.1
2U	44	5.0	−1.9	<0.001	6.5	27.0	33	32.2	10.3	10.3	30.8	−1.8	0.1
2V	43	4.4	−0.4	<0.001	5.3	36.8	26	25.8	11.4	11.5	22.5	−3.4	−0.2
2W	37	5.8	−14.0	<0.01	6.3	39.3	31	29.0	14.0	14.7	22.5	−8.8	0.1
2Y	82	0.6	7.8		8.2	63.8	42	24.9	32.5	23.3	12.5	−29.1	0.0
3A	131	6.6	−7.7	<0.001	8.0	62.1	40	46.3	16.9	16.0	45.2	4.8	0.2
3B	42	5.7	4.9	<0.001	6.4	88.3	32	41.4	13.8	14.0	41.4	9.4	9.8
4G	13	6.3	−5.0	<0.01	12.8	117.0	66	76.0	26.5	22.1	76.0	10.1	0.3
ALL	3226	5.3	−2.0	<0.001	5.2	55.3	26	33.2	17.8	15.2	26.9	−0.2	0.1

Note: Data are the subset of that shown in Table 1 for which required chemistry data were available. Analyses were performed for individual secondary watersheds, and for all 3226 records together. Slope and intercept and probability of a regression of alkalinity residuals ($[Alk]_{r,estimated} - [Gran\ Alk]_{r,observed}$) on DOC concentration are shown. Also shown are median DOC and median observed alkalinity, as well as the value of the regional correction factor (CF) applied based on the secondary watershed-specific median DOC. Residuals show fit and bias without a CF, with a regional CF (reg. CF), and with a CF specific to each secondary watershed (WS CF). Avg. abs. resid., average absolute residual.

watershed basis and for the entire data set. There is theoretical justification for a linear approach (Hemond 1990). The regression of alkalinity residuals on DOC was significant at $p < 0.001$ for 15 of 25 secondary watersheds studied and also for the entire data set (Table 2). The median regional correction factor (based on DOC) is always greater than 15 μequiv./L (Table 1), which has significant implications for pH and biological predictions. A 26-μequiv./L decrease in ANC (median correction factor) produces an average decrease in pH of 0.1–0.4 pH units for the 25 secondary watersheds. Some watersheds would show much larger shifts in pH when the correction factor is applied.

The correction factor substantially improves the fit and reduces the overall bias in Gran Alk estimates. Applying either a regional or watershed-specific correction factor halves the average absolute value of residuals compared with no correction factor (bottom row of Table 2, fit columns). The watershed-specific correction factor does not much improve the fit over the regional correction factor. The bias of the residuals is naturally reduced as the regressions are more watershed specific (Table 2, bias columns).

On the basis of this analysis, we modified the ESSA–DFO model to routinely subtract the regional correction factor, 5.3(DOC) – 2.0, from alkalinity estimates. This is in close agreement with Hemond (1990) who showed through simulated titrations that $(C_B - C_A)Alk = Gran\ ANC + 4.6(DOC)$, for waters in equilibrium with the atmosphere ($P_{CO_2} = 10^{-3.5}$). The coefficient of DOC in the correction factor, 5.3, again represents the estimated charge density in microequivalent anions per milligram DOC. This estimate is more consistent with other studies (Table 3) than the estimate we derived from titration curve fits.

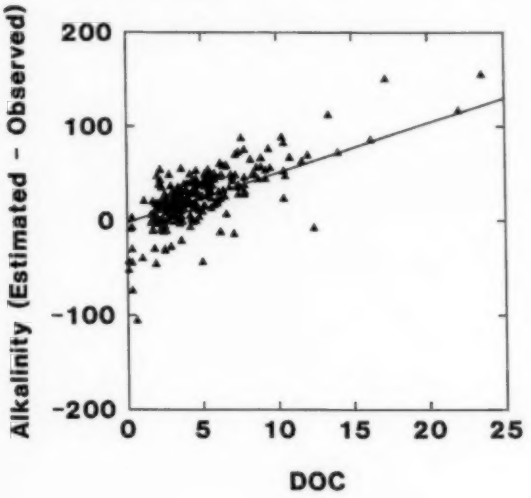
The corrections described in this paper can significantly change regional model predictions. For example, when we included the new titration curve and correction factor in the ESSA–DFO acidification model, we obtained a closer correspondence between predicted and observed current Gran Alk, and lower estimates of original (pre-industrial) and eventual ANC (reduced by correction factor). Though there is no change in the estimated ANC decline since pre-industrial times (we assume constant DOD), the regional pH distributions are shifted towards more toxic levels. These modifications are particularly important in estimating acid precipitation effects on high DOC surface waters frequented by fish, waterfowl, and other biota whose reproductive success and probability of presence are strongly related to pH levels (Kelso et al. 1986; Baker et al. 1990; Blancher et al. 1992; McNicol et al. 1995). The new titration curve and DOC correction factor apply to

© 1996 NRC Canada

Table 3. Charge density estimates from other studies (adapted from Wilkinson et al. 1992).

Region	Mean charge density (μequiv./mg DOC)	Source
Massachusetts	7.5	Eshleman and Hemond 1985
Finland	7.2	Kortelainen 1992
Quebec	6.0–8.1	Wilkinson et al. 1992
Norway, Scotland	5.5	Henriksen and Seip 1980
Norway	4–5	Brakke et al. 1987
Norway	<4.5	Wright 1989
Nova Scotia	4.8	Gorham et al. 1985
Maine	4.5	Kahl et al. 1989
Eastern Canada	5.3	This study (correction factor)
Eastern Canada	1.9–2.3	This study (titration curve)
Minnesota	2.6–10.1	Urban et al. 1989
Adirondacks	2.1	Driscoll and Schafran 1984

Fig. 3. Alkalinity residuals ($[CBANC]_{t,estimated} - [Gran\ Alk]_{t,observed}$) plotted versus DOC, for a 250-point random subsample of the 3226 records with complete chemical information. The regression line of residuals on DOC for the entire 3226-point data set is shown (see Table 2 for numerical values).



secondary watersheds in eastern Canada south of 52°N. If the approach is to be used outside the present region of study, the titration curve parameters and correction factor should be fit to data from the new area.

Acknowledgments

The data used in these analyses were supplied, with the permission of their respective agencies, by B. Neary, Ontario Ministry of the Environment and Energy; D. Jeffries, Environment Canada; D. McNicol, Canadian Wildlife Service (Ontario Region); and J. Dupont, ministère de l'Environnement du Québec. The Canadian Wildlife Service provided the funding for this study under the federal Long Range Transport of Air Pollutants program. We thank two anonymous reviewers for their thoughtful suggestions.

References

Baker, J.P., Bernard, D.P., Christensen, S.W., Sale, M.J., Freda, J., Heltcher, K.J., Marmorek, D.R., Rowe, L., Scanlon, P.F., Suter II, G.W., Warren-Hicks, W.J., and Welbourn, P.M. 1990. Biological effects of changes in surface water acid–base chemistry. NAPAP report No. 13. *In* Acidic deposition: state of science and technology. Vol. 2. National Acid Precipitation Assessment Program, Washington, D.C.

Blancher, P.J., McNicol, D.K., Ross, R.K., Wedeles, C.H.R., and Morrison, P. 1992. Towards a model of acidification effects on waterfowl in eastern Canada. Environ. Pollut. **78**: 57–63.

Brakke, D.F., Henriksen, A., and Norton, S.A. 1987. The relative importance of acidity sources for humic lakes in Norway. Nature (London), **329**: 432–434.

Bulger, A.J., Lien, L., Cosby, B.J., and Henriksen, A. 1993. Brown trout (*Salmo trutta*) status and chemistry from the Norwegian Thousand Lake Survey: statistical analysis. Can. J. Fish. Aquat. Sci. **50**: 575–585.

Clair, T.A., Pollock, T.L., Collins, P.V., and Kramer, J.R. 1992. How brown waters are influenced by acidification: the Humex Lake case study. Environ. Int. **18**: 589–596.

Cosby, B.J., Bulger, A.J., and Wright, R.F. 1994. Predicting recovery of freshwater ecosystems: trout in Norwegian lakes. *In* Acidification of freshwater ecosystems: implications for the future. *Edited by* C.E.W. Steinberg and R.F. Wright. John Wiley & Sons, New York. pp. 355–373.

Driscoll, C.T., and Schafran, R.C. 1984. Short-term changes in the base neutralizing capacity of an acid Adirondak lake, New York. Nature (London), **310**: 308–310.

Dupont, J. 1993. Québec lake survey: status of lake acidification in Québec. Envirodoq No. EN920000, Rep. No. QEN/PA-47a/1. Ministère de l'Environnement du Québec, Direction de la qualité du milieu aquatique, Québec, Que.

Eshleman, K.N., and Hemond, H.F. 1985. The role of organic acids in the acid–base status of surface waters at Bickford watershed, Massachusetts. Water Resour. Res. **21**: 1503–1510.

Federal–Provincial Research and Monitoring Coordinating Committee. 1990. The 1990 Canadian long-range transport of air pollutants and acid deposition assessment report. Part 4. Aquatic effects. Environment Canada, Downsview, Ont.

Gorham, E., Eisenreich, S.J., Ford, J., and Santelmann, M.V. 1985. The chemistry of bog waters. *In* Chemical processes in lakes. *Edited by* W. Stumm. John Wiley & Sons, New York. pp. 339–363.

Hemond, H.F. 1990. Acid neutralizing capacity, alkalinity, and acid–base status of natural waters containing organic acids. Environ. Sci. Technol. **24**: 1486–1489.

Henriksen, A., and Seip, H.M. 1980. Strong and weak acids in surface

© 1996 NRC Canada

waters of southern Norway and southwestern Scotland. Water Res. **14**: 809–813.

Jones, M.L., Minns, C.K., Marmorek, D.R., and Elder, F.C. 1990. Assessing the potential extent of damage to inland lakes in eastern Canada due to acidic deposition. II. Application of the regional model. Can. J. Fish. Aquat. Sci. **47**: 67–80.

Kahl, J.S., Norton, S.A., Macrae, R.K., Haines, T.A., and Davis, R.B. 1989. The influence of organic acidity on the acid–base chemistry of surface waters in Maine, U.S.A. Water Air Soil Pollut. **46**: 221–233.

Kamari, J., Brakke, D.F., Jenkins, A., Norton, S.A., and Wright, R.F. (Editors). 1989. Regional acidification models: geographic extent and time development. Springer-Verlag, Heidelberg, Germany.

Kelso, J.R.M., Minns, C.K., Gray, J.E., and Jones, M.L. 1986. Acidification of surface waters in eastern Canada and its relationship to aquatic biota. Can. Spec. Publ. Fish. Aquat. Sci. No. 87.

Korman, J., Marmorek, D.R., and Wedeles, C.H.R. 1991. Analysis of the effects of dissolved organic carbon and lake size bias on waterfowl acidification response projections. Prepared for Canadian Wildlife Service, Hull, Que.

Kortelainen, P. 1992. Charge density of total organic carbon in Finnish lakes. Environ. Pollut. **77**: 107–113.

Marmorek, D.R., Jones, M.L., Minns, C.K., and Elder, F.C. 1990. Assessing the potential extent of damage to inland lakes in eastern Canada due to acidic deposition. I. Development and evaluation of a simple "site" model. Can. J. Fish. Aquat. Sci. **47**: 55–66.

McNicol, D.K., Mallory, M.L., and Wedeles, C.H.R. 1995. Assessing biological recovery of acid-sensitive lakes in Ontario, Canada. Water Air Soil Pollut. **85**: 457–462.

Minns, C.K., Moore, J.E., Schindler, D.W., and Jones, M.L. 1990. Assessing the potential extent of damage to inland lakes in eastern Canada due to acidic deposition. III. Predicted impacts on species richness in seven groups of aquatic biota. Can. J. Fish. Aquat. Sci. **47**: 821–830.

Neary, B.P., Dillon, P.J., Munro, J.R., and Clark, B.J. 1990. The acidification of Ontario lakes: an assessment of their sensitivity and current status with respect to biological damage. Limnology Section, Ontario Ministry of Environment, Dorset Research Centre, Dorset, Ont.

Perdue, E.M. 1978. Solution thermochemistry of humic substances. I. Acid–base equilibria of humic acid. Geochim. Cosmochim. Acta, **42**: 1351–1358.

Schnoor, J.L., Palmer, W.D. Jr., and Glass, G.E. 1984. Modeling impacts of acid precipitation for northeastern Minnesota. *In* Modeling of total acid precipitation impacts. *Edited by* J.L. Schnoor. Butterworth Publishers, Stoneham, Mass. pp. 155–173.

Small, M.J., and Sutton, M.C. 1986. A regional pH–alkalinity relationship. Water Res. **20**: 335–343.

Sullivan, T.J., Driscoll, C.T., Cook, R.B., Gherini, S.A., Charles, D.F., and Yatsko, C.P. 1989. The influence of organic acid anions and aqueous aluminum on measurements of acid neutralizing capacity in surface waters. Nature (London), **338**: 408–410.

Thompson, M.E. 1982. The cation denudation rate as a quantitative index of sensitivity of eastern Canadian rivers to acidic atmospheric precipitation. Water Air Soil Pollut. **18**: 215–226.

Thornton, K.W., Marmorek, D., Ryan, P.F., Heltcher, K., and Robinson, D. 1990. Methods for projecting future changes in surface water acid–base chemistry. Acidic deposition; state of science and technology. Report No. 14. National Acid Precipitation Assessment Program, Washington, D.C.

Urban, N.R., Bayley, S.E., and Eisenreich, S.J. 1989. Export of dissolved organic carbon and acidity from peatlands. Water Resour. Res. **25**: 1619–1628.

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Ill.

Wilkinson, K.J., Jones, H.G., Campbell, P.G.C., and Lachance, M. 1992. Estimating organic acid contributions to surface water acidity in Quebec (Canada). Water Air Soil Pollut. **61**: 57–74.

Wright, R.F. 1989. RAIN project: role of organic acids in moderating pH change following reduction in acid deposition. Water Air Soil Pollut. **46**: 251–259.

Wright, R.F., and Henriksen, A. 1983. Restoration of Norwegian lakes by reduction in sulphur deposition. Nature (London), **305**: 422–424.

© 1996 NRC Canada

Effect of alewife predation on survival of larval yellow perch in an embayment of Lake Ontario

Doran M. Mason and Stephen B. Brandt

Abstract: We examined the effect of alewife predation on larval yellow perch between 1984 and 1986 in North Pond and South Pond, embayments of Lake Ontario. The ponds provided a natural experiment; larval yellow perch occurred in both ponds, but alewife occurred only in North Pond. Alewife consumed yellow perch larvae during late April and early May in North Pond in 1985 and 1986. In 1984, alewife immigrated into North Pond 3 weeks after the peak hatch of yellow perch and predation was not observed. Instantaneous daily mortality rates of larval yellow perch were higher in North Pond in 1985 (1.58) than in South Pond in all 3 years (0.22–0.28) and coincided with the highest alewife abundance of the 3 years. Temperature, wind events, and food availability could not directly account for the high larval mortality rate. A predator–prey model further supported our findings; alewife predation was sufficient to account for the high larval mortality rate. Predation by alewife appears to be a source of significant larval yellow perch mortality in North Pond and could effect the recruitment of yellow perch in Lake Ontario.

Résumé : Nous avons examiné l'effet de la prédation exercée par le gaspareau (*Alosa pseudoharengus*) sur les larves de perchaude (*Perca flavescens*) entre 1984 et 1986 dans deux anses du lac Ontario, North Pond et South Pond. Les anses ont constitué des laboratoires à ciel ouvert; les larves de perchaude se retrouvaient dans les deux anses, mais le gaspareau seulement dans North Pond. Le gaspareau a consommé les larves de perchaude à la fin avril et au début mai dans North Pond en 1985 et 1986. En 1984, les gaspareaux sont entrés dans North Pond 3 semaines après le pic d'éclosion de la perchaude, et on n'a pas observé de prédation. Les taux quotidiens instantanés de mortalité des larves de perchaude étaient plus élevés dans North Pond en 1985 (1,58) que dans South Pond au cours des 3 années (0,22–0,28), et coïncidaient avec l'abondance de gaspareau la plus élevée des 3 années. La température, les épisodes de vent et la disponibilité de la nourriture ne permettaient pas d'expliquer directement le taux élevé de mortalité des larves. Un modèle de la prédation est venu étayer nos résultats; la prédation exercée par le gaspareau suffisait à expliquer la forte mortalité des larves. Cette prédation semble être la cause d'une mortalité importante des larves de perchaude dans North Pond, et pourrait avoir un effet sur le recrutement de la perchaude dans le lac Ontario.
[Traduit par la Rédaction]

Introduction

The inverse relationship between yellow perch (*Perca flavescens*) year-class strength and abundance of alewife (*Alosa pseudoharengus*) indicates that alewife may reduce yellow perch recruitment (Smith 1970; Wells 1977; Crowder 1980; Jude and Tesar 1985; Eck and Wells 1987). The strongest correlations have been found in Lake Michigan, which experienced a decrease in juvenile yellow perch abundance, a collapse in the inshore yellow perch sport fishery, and an abrupt decline in the commercial harvest of yellow perch coincident with the rapid increase of alewife (Wells 1977). In recent years, year-class strength of yellow perch in Lake Michigan continued to inversely fluctuate with alewife abundance (Eck and Wells 1987). Yellow perch numbers increased fivefold following the collapse in alewife abundance (86% reduction) in the early 1980s (Jude and Tesar 1985; Eck and Wells 1987).

The mechanism of this potential species interaction is not clear. Alewife may compete with the early life stages of yellow perch for food (Crowder 1980; Crowder et al. 1987), alewife may prey on the larvae of yellow perch (Crowder 1980; Jude and Tesar 1985; Eck and Wells 1987), or both (Mason 1989). Alewife are predators of larval yellow perch (Kohler and Ney 1980; Brandt et al. 1987), and predation is believed to be the mechanism by which alewife affect yellow perch recruitment (Crowder 1980). However, the significance of alewife predation on total larval mortality and year-class strength of yellow perch is unknown.

Predation by alewife on yellow perch larvae should be most intense during periods of high spatial and temporal overlap. In Lake Ontario, this overlap occurs in the spring when both species are in shallow embayments and tributaries (Brandt et al. 1987). In addition, predation may be size dependent. Brandt et al. (1987) found that yellow perch larvae less than 8.0 mm were most vulnerable to alewife predation. Therefore, a narrow temporal window may exist when predation by alewife will have the greatest effect on yellow perch survival.

This paper extends our preliminary observations that documented predation by alewife on larval yellow perch in an embayment of Lake Ontario (Brandt et al. 1987). Here, we set out to determine if predation by alewife on larval yellow perch could be sufficient to cause a decline in larval abundance. Our

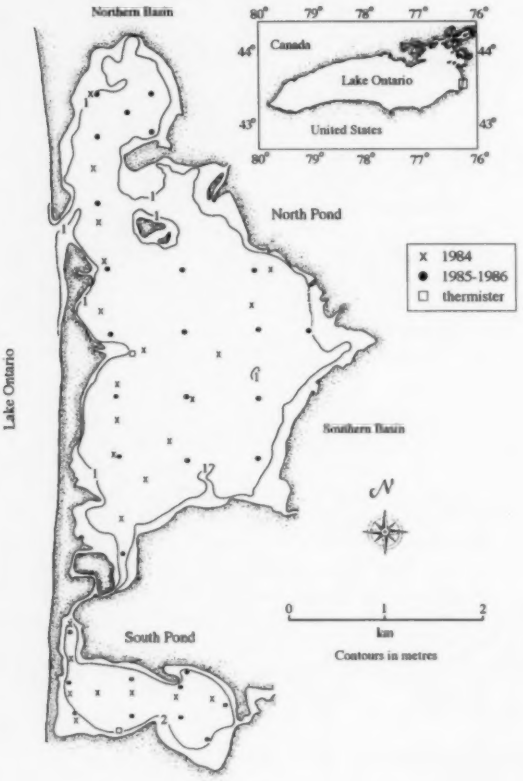
Received June 28, 1994. Accepted January 19, 1996.
J12455

D.M. Mason.[1] Center for Limnology, University of Wisconsin, 680 North Park Street, Madison, WI 53706–1492, U.S.A.
S.B. Brandt. Great Lakes Center, State University of New York College at Buffalo, 1300 Elmwood Avenue, Buffalo, NY 14222, U.S.A.

[1] Author to whom all correspondence should be addressed.
e-mail: mason@limnosun.limnology.wisc.edu

© 1996 NRC Canada

Fig. 1. Map of North Sandy Pond and South Sandy Pond showing the larval perch sampling sites used in 1984–1986 and the location of the continuously recording thermisters.



approach was to (*i*) document the timing and magnitude of larval mortality relative to the timing and magnitude of the inshore spawning migration of alewife, (*ii*) compare larval mortality rates between two ponds (with and without the presence of alewife) and among years (variable timing of alewife migration and abundance), and (*iii*) use a predation model to evaluate the affect of alewife predation on the mortality rate of larval yellow perch.

Study sites

Our study sites were North Sandy Pond (North Pond) and South Sandy Pond (South Pond). The embayments are on the eastern shore of Lake Ontario adjacent to Mexico Bay in New York state (Fig. 1). Both ponds are important spawning and nursery areas for eastern Lake Ontario yellow perch populations (Storr 1977). However, alewife spawn only in North Pond.

North Pond has a surface area of about 988 ha, a maximum depth of 5 m, a relatively narrow and shallow opening to Lake Ontario, and two distinct basins (Fig. 1). The northern basin (~137 ha) contains submergent vegetation whereas the southern basin (North Pond proper, ~851 ha) is mostly devoid of vegetation. South Pond is smaller (82 ha) and deeper (maximum depth 7 m) than North Pond and has submergent vegetation around its perimeter. South Pond has no direct access to Lake Ontario. A narrow and shallow canal (<1 m) connects North and South ponds (Fig. 1). Fish species composition is similar in North and South ponds and characterized by a centrachid–percid community (D.M. Mason, unpublished data). The two ponds differ only in the presence of alewife (only in North Pond) and lake morphology.

Methods

Field data collection

Larval yellow perch and adult alewife were sampled in North and South ponds during 1984 (April 25 – June 14), 1985 (April 15 – June 11), and 1986 (April 26 – May 14). Sampling was conducted almost daily in 1984 and 1985 and weekly in 1986. Sampling design and procedure varied according to year. For all years, larval yellow perch were collected during day and night with oblique tows (near bottom to surface) of a 0.62 m diameter bongo sampler (McGowan and Brown 1966) fitted with two 0.243-mm mesh Nitex nets. Nets were retrieved at 0.9–1.1 m/s. Immediately after retrieval, nets were rinsed and contents stored in 5% buffered formalin. A General Oceanics flowmeter, mounted on the bongo sampler, was used to calculate the volume of water filtered. Fish larvae were sorted, counted, identified using the keys of Auer (1982), and measured to the nearest 0.1 mm total length (TL). In 1984, larval fish were randomly sampled from stations equally spaced along two transects of the major and minor axes in North and South ponds (Fig. 1). In 1985 and 1986, a stratified random grid design was implemented in both North and South ponds primarily to increase the number of sampling stations in the northern basin of North Pond from one to five (Fig. 1).

For all years, alewife were sampled during day and night in North and South ponds using a bottom trawl (7.6-m headrope, 2-cm bar mesh, and 0.6-cm mesh liner) and a 1-m^2 framed midwater trawl (1-cm mesh). Alewife collected in bottom trawls were used as an index of abundance and for stomach analyses. Alewife collected in midwater trawls were used only for stomach analyses. Trawls were towed for 5–10 min at 0.9–1.1 m/s. In 1986, alewife were also collected from the northern basin of North Pond using monofilament gill nets (25.5–63.5 mm square meshes, 1.8 m deep panels) set for approximately 1 h at the surface shortly after sunset. Upon capture, the body cavities of alewife were opened immediately and whole fish were preserved in 10% buffered formalin.

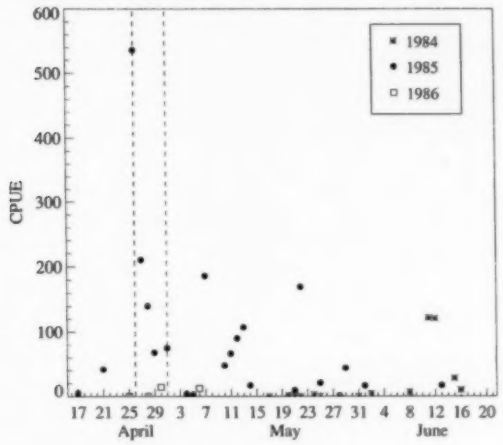
In the laboratory, alewife were measured to the nearest 1 mm TL, stomachs were removed, and stomach contents were examined using a dissecting microscope. All fish larvae were identified and counted. Partially digested larvae were identified by examining body fragments and pigmentation. Yellow perch were the only fish species found in the stomachs of alewife. Furthermore, yellow perch were the only fish larvae collected in the bongo nets during the period when predation was observed.

Temperature profiles were taken almost daily in both ponds during the spring of 1984 and 1985, using a YSI thermister. In 1985, a continuously recording thermistor was suspended 1 m below the surface of the water in both ponds (Fig. 1).

Total instantaneous daily mortality rate (Z) was calculated for larval yellow perch by fitting larval density data (determined from bongo net catches and volume of water filtered) to the equation $N_t = N_0 e^{-Zt}$ using nonlinear regression (Marquardt method; SAS Institute Inc. 1988), where N_t is the number of larval perch at time t (days) and N_0 is the initial larval density. Larval density data were fitted with a nonlinear regression model using those data collected on and after the date of the peak density of yellow perch. Maximum observed mean density was used to establish the timing of peak larval density.

© 1996 NRC Canada

Fig. 2. Catch per unit effort (CPUE) of alewife in North Sandy Pond between 1984 and 1986. Effort is number per 10-min tow. Larval yellow perch first appeared between April 26 and May 1 for all years (vertical broken lines).



Predation model

We evaluated the role of alewife predation by setting Z in the above equation to $m_n + m_p$ (where m_n is mortality from causes other than predation by alewife, and m_p is predation mortality) and solving for the number of larvae surviving the first 10 days of life. The values for m_p were calculated by forcing $N_1 = N_0 - AP$, where A is the abundance of alewife (numbers) and P is the predation rate in number of larvae eaten per alewife per day, setting $m_n = 0$, and solving for $m_p = \ln N_0 - \ln N_1$. N_0 was set at 151 million, the estimated abundance of yellow perch larvae in the northern basin of North Pond in 1985 (Mason 1989). The 10-day temporal window was determined as the length of time required for larvae to grow out of the most vulnerable stage, >8.0 mm (Brandt et al. 1987) assuming a growth rate of 0.2 mm/day (Mason 1989). Natural mortality, alewife abundance, and alewife predation rate were systematically varied to determine the various combinations of mortalities possible for comparison with observed total mortality of larvae. Values used for natural mortality (m_n) were varied from 0.0 to 0.3. The range of values used for P was determined from the stomach content data of alewife.

Results

The highest concentration of alewife occurred in the northern basin of North Pond during dusk and at night (Brandt et al. 1987). Furthermore, the northern basin also contained the greatest concentrations and abundance of larval yellow perch (Brandt et al. 1987). Thus, results will emphasize alewife and larval yellow perch interactions occurring in the northern basin on North Pond.

In all years, yellow perch larvae first appeared in North Pond samples between April 26 and May 1 (Figs. 2 and 3). In contrast, the first appearance of alewife in North Pond varied between years. In 1984, alewife were first caught in North Pond on May 20, approximately 3 weeks following the emergence of yellow perch larvae; peak abundance of alewife did not occur until mid-June, approximately 6 weeks post-emergence. During 1985 and 1986, alewife appeared in North Pond coincident with the emergence of yellow perch during the last week in April. Alewife abundance in North Pond also varied among years (Fig. 2). The greatest overall catch per unit effort of alewife (CPUE; number per 10-min tow) occurred in 1985 (mean = 85.6, n = 183), followed by 1984 (mean = 22.7, n = 72), with the lowest abundance of alewife occurring in 1986 (mean = 6.5, n = 17). In 1985, alewife had higher densities prior to and during larval emergence. In 1986, the first appearance of alewife coincided with larval emergence, but overall, alewife abundances were the lowest of the 3 years. No alewife were captured in South Pond during the 3 years of this study.

In 1985, larval yellow perch abundance in the northern basin of North Pond declined by 80% during the first 24-h period and 99% by the 10th day (Fig. 3). Furthermore, larval yellow perch were rare in collections from the southern basin of North Pond, and after May 15 they did not occur in bongo samples. The rapid decline in larval abundance coincided with the highest alewife abundance observed during the 3 years of the study. A second larval emergence occurred on May 8 in the northern basin of North Pond, but this was followed by another rapid decline in larval abundance. A decline of the magnitude observed in North Pond 1985 did not occur in North Pond in 1984 and 1986 nor in South Pond for any year (Fig. 3).

Mean total length of larval yellow perch (±95% confidence interval) from North Pond showed no significant increase between April 30 (5.98 ± 0.03 mm, n = 190) and May 15, 1985 (6.15 ± 0.42 mm, n = 10). Larvae from South Pond increased in length from 5.80 ± 0.03 mm (n = 317) to 9.21 ± 0.09 mm (n = 108) during the same period (Fig. 4). Larval lengths from all other years of the study showed a significant increase over time (Fig. 4).

Alewife predation on yellow perch occurred between April 30 and May 5 in the northern basin of North Pond in 1985 during the abrupt larval decline. Thirty percent of alewife stomachs (n = 40) contained yellow perch. Percent occurrence was as high as 70% (n = 13) on April 30 (Fig. 5). The mean number of larvae found per alewife stomach was 3.8 (range 0–6).

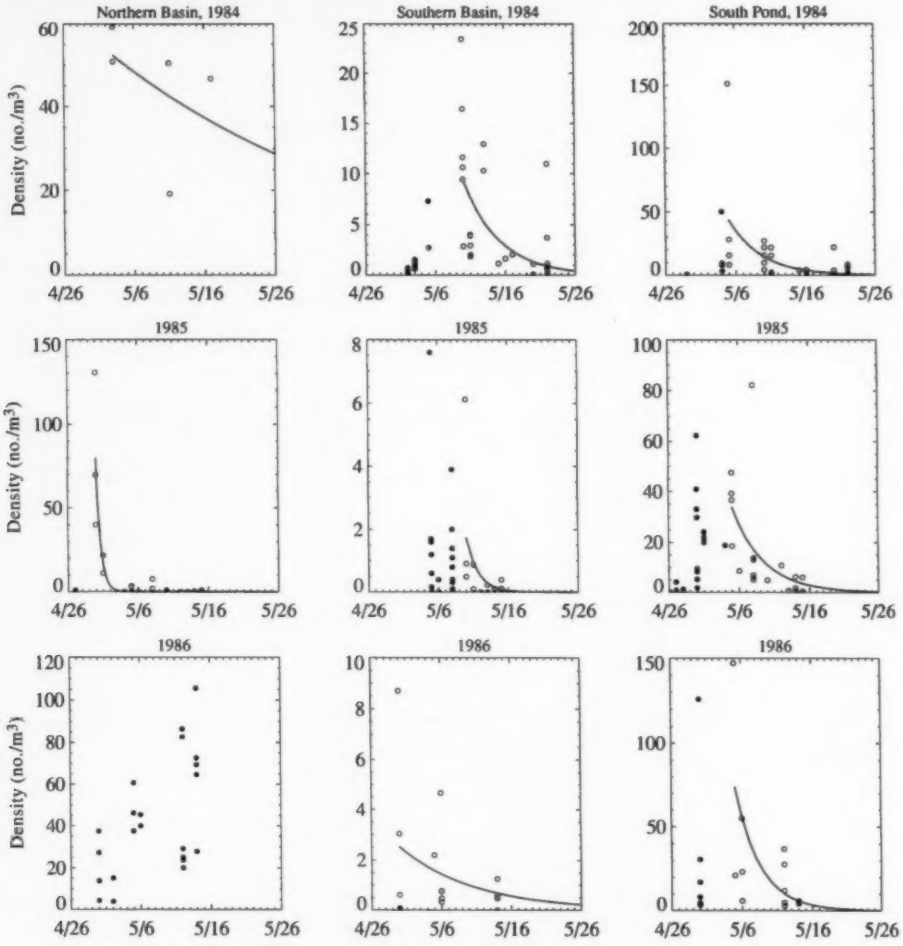
Unlike the findings in 1985, the mean density of larval yellow perch in 1986 increased from 21 to 50 larvae/m^3 in the northern basin of North Pond. On average, 41% of the alewife stomachs examined (n = 71) from the northern basin of North Pond contained larval yellow perch. The proportion of alewife consuming larvae increased from 38 to 78% between 30 April and 14 May (Fig. 5). Of the alewife that did not have empty stomachs (n = 41), larval occurrence increased from 63% on April 30 to 100% on May 14. Overall, the mean number of larvae consumed per alewife was 33, increasing from 24 on April 30 to 171 on May 14 (range 0–361). Sixty-four percent of the alewife that consumed yellow perch contained only yellow perch, whereas the remaining alewife also consumed chironomid larvae, *Chaoborus* larvae, and gammarids.

Estimates of the instantaneous daily mortality rate of larvae ranged from 0.03 to 1.58 (Table 1). High variability in larval catch made it difficult to statistically discern differences in mortality rates between sampling basins, ponds, and years (Table 1). However, estimates of the instantaneous daily mortality rate of yellow perch in the northern basin of North Pond were 60 times higher in 1985 than in 1984 and 6–7 times higher than those in South Pond in all years (Table 1).

The predator–prey model evaluates the interactive effect of alewife predation rates, alewife abundances (Fig. 6A), and larval

© 1996 NRC Canada

Fig. 3. Densities of larval yellow perch in the northern and southern basins of North Pond and in South Pond between 1984 and 1986. Open circles are data used to fit the nonlinear regression model and solid circles are all other data.



natural mortality (Fig. 6B) on larval total mortality. Given the range of natural mortality rates estimated for North Pond in 1984 and 1986 and for South Pond (the control) in all years (range 0.03–0.28), alewife predation would need to account for an instantaneous daily mortality rate of 0.17–0.43 to result in the observed 10-day decline (≥99%) in larval abundance. However, in the northern basin of North Pond during 1985, m_p ranged from 0.98 to 1.55.

Water temperature and daily fluctuations in water temperature were similar between North and South ponds in 1985 (Fig. 7). Prior to larval emergence (April 18 – April 29), water temperature increased 0.5°C/day in both North and South ponds (Fig. 7). Yellow perch emerged when the water temperature attained 11.4°C in North Pond and 12.0°C in South Pond; water temperature remained relatively constant from April 30 to May 10 and then increased at a rate of 0.25°C/day in North Pond and 0.27°C/day in South Pond (Fig. 7). Severe storms and high winds only occurred on May 6–7 and 12 in 1985, 4 and 8 days after the period of greatest larval mortality (Fig. 5).

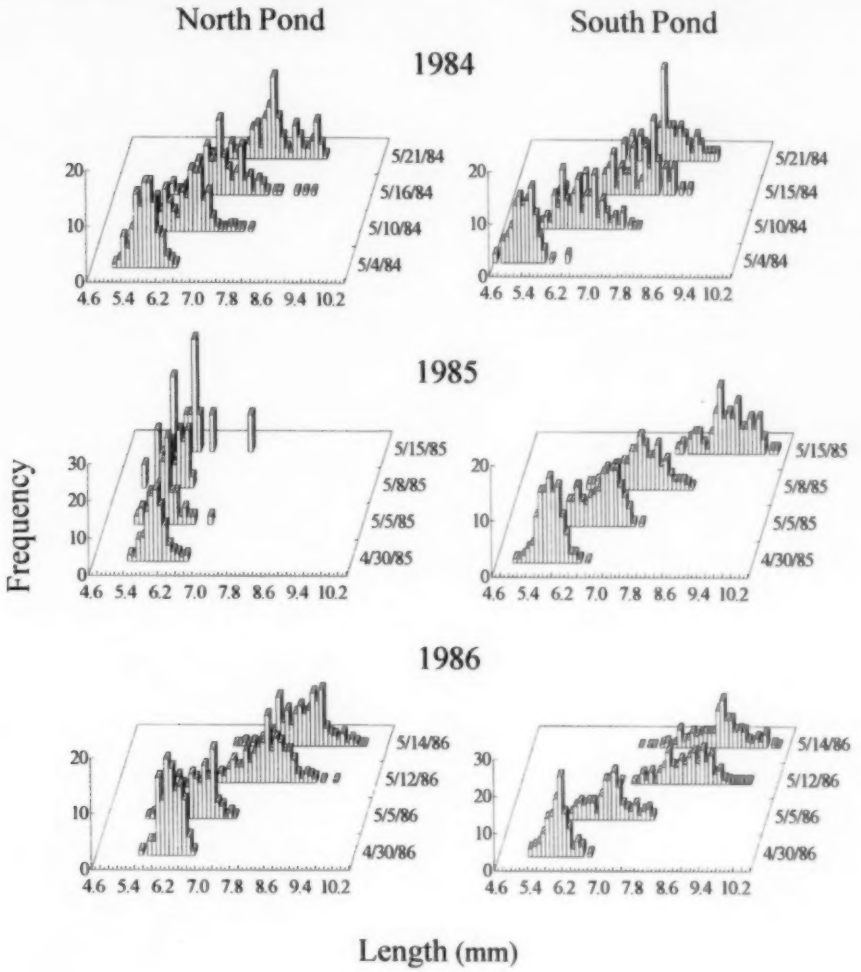
The mean daily water temperature in 1984 was similar to that observed in 1985 from April 26 to May 12 (Fig. 7). However, before April 26, water temperatures in 1984 were higher than in 1985. After May 12, North and South ponds in 1984 did not undergo a warming trend as observed in both ponds during 1985.

Discussion

A 10- to 50-fold annual variation in yellow perch year-class strength is not unusual (Hile and Jobes 1940; Doan 1942; Forney 1976; Eshenroder 1977; Hamely et al. 1983; Henderson 1985). The mechanisms regulating year-class strength for yellow perch are believed to operate during the early life history stages (Clady 1976; Forney 1971; Mills and Forney 1981). Attempts to correlate environmental conditions directly to

© 1996 NRC Canada

Fig. 4. Length–frequency distribution of larval yellow perch in North Pond and South Pond between 1984 and 1986.



year-class strength have generally been unsuccessful (Doan 1942; Clady 1976; Koonce et al. 1977) except under extreme conditions and localized cases (Eshenroder 1977; Henderson 1985; Newsome and Aalto 1987). Others have suggested that interannual differences in predation rates and food availability during the early life stages of yellow perch affect recruitment (Forney 1971, 1977; Crowder 1980; Nielson 1980). Recent evidence suggests that small changes in a single factor affecting mortality (e.g., predation) may affect the success of a year-class (Houde 1987).

Our preliminary results (Brandt et al. 1987) clearly demonstrated that individual alewife can consume a significant number of emerging larval yellow perch (>100) in a single evening and alewife predation on larvae occurs principally at dusk. Unquestionably, alewife predation does contribute to larval mortality; however, the magnitude of this predation remains to be evaluated. Our field experiment addressed this question of alewife effects on the mortality rate of larval yellow perch. We had 3 years of field sampling in two ponds, one pond containing alewife (North Pond) and one pond without alewife (South Pond). In addition, the timing and magnitude of the alewife migration into North Pond varied during the 3 years of the study. The timing of larval emergence was similar among years, i.e., late April. Using larval emergence as a time reference, the appearance of alewife was either early (before or during larval emergence in 1984 and 1986) or late (well after larval emergence in 1985). Moreover, the magnitude of the alewife migration varied between years. Thus, the naturally occurring events provided the following contrasts: early migration and high alewife abundance (1985), early migration and low alewife abundance (1986), and late migration and high alewife abundance (1984). The variation in timing and magnitude of the alewife migration combined with our control site provided a naturally occurring experiment.

Alewife predation on larval yellow perch should have been most pronounced when alewife abundance was high and alewife

© 1996 NRC Canada

Table 1. Estimates of the instantaneous daily mortality coefficients for larval yellow perch (Z) and regression statistics from field samples collected in the northern basin of North Pond (NB), the southern basin of North Pond (SB), and South Pond between 1984 and 1986.

Pond	Year	Site	n^a	Z	95% confidence limits		r^2
					Upper	Lower	
North Pond	1984	NB	5	0.026	0.123	−0.071	0.19
		SB	26	0.196	0.421	−0.029	0.28
	1985	NB	22	1.580	2.925	0.236	0.78
		SB	15	0.601	1.758	−0.557	0.36
	1986	NB[b]	—	—	—	—	—
		SB	15	0.094	0.303	−0.114	0.11
South Pond	1984		30	0.221	0.434	0.009	0.27
	1985		20	0.225	0.463	−0.013	0.32
	1986		13	0.283	0.658	−0.091	0.42

[a]Number of paired bongo tows.
[b]Larvae never attained peak abundance.

Fig. 5. Mean larval density and percent frequency of occurrence of larvae in alewife stomachs in the northern basin of North Pond in 1985 and 1986.

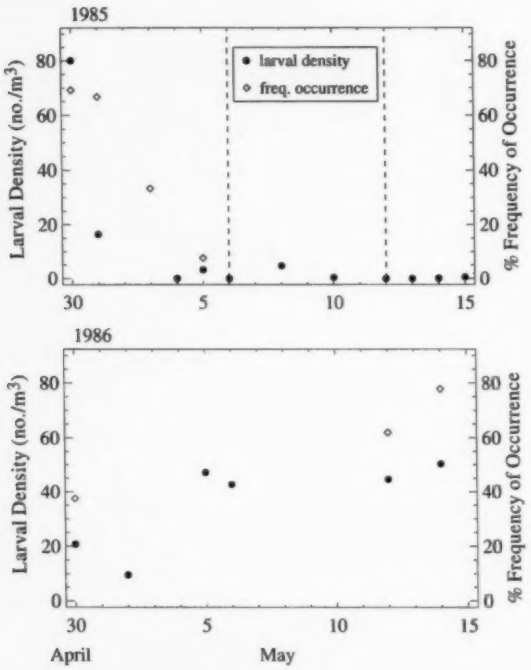
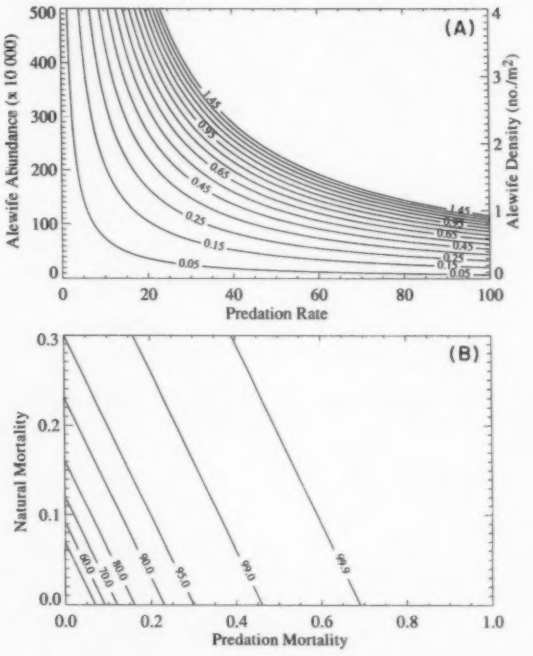


Fig. 6. (A) Yellow perch mortality isoclines (instantaneous daily predation mortality rate) given predation rate (number of larvae consumed per alewife per day) and alewife abundance. The right vertical axis represents the corresponding mean alewife density for the northern basin of North Pond given alewife abundance on the left vertical axis. (B) Percent mortality of larval yellow perch at the end of 10 days as a function of instantaneous daily predation mortality rate (from above) and other sources of instantaneous daily mortality.

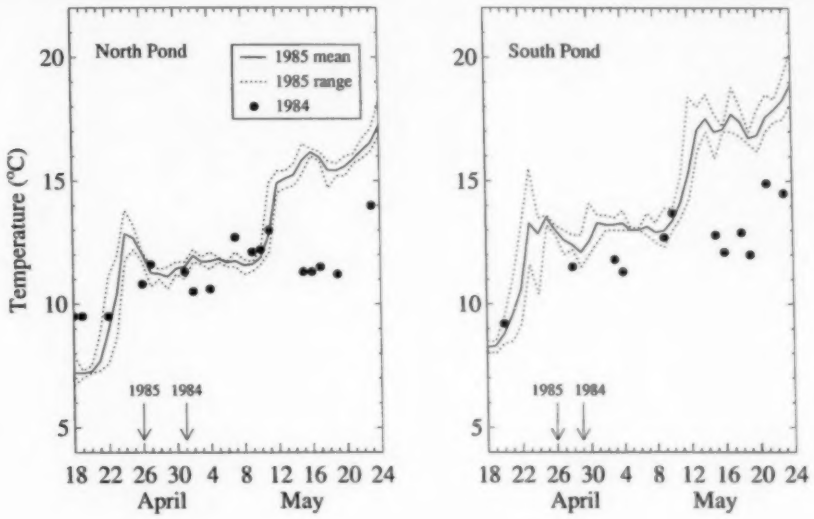


were present during the emergence of yellow perch. The most rapid decline and highest estimated instantaneous daily mortality rate ($Z = 1.58$) of larval yellow perch occurred in North Pond during 1985. The mortality rate of yellow perch was lower in South Pond during all years and in North Pond during 1984 and 1986. Larval mortality rates in North Pond in 1984 and 1986 were relatively low (Table 1), despite the early alewife inshore migration and known alewife piscivory in 1986. However, alewife abundance in 1986 was the lowest of the 3 years. Length–frequency data further support our conclusions that the larval yellow perch mortality rate was higher than average in 1985 because few larvae survived beyond the prolarval stage. Larvae increased in length in all other years in North Pond and for all years in South Pond.

Alewife predation can account for a significant proportion of the total Z of larval yellow perch. If larval mortality rates

© 1996 NRC Canada

Fig. 7. Mean daily water temperature and range from a continuously recording thermister for North Pond and South Pond in 1985. Solid circles are mean water temperatures recorded in 1984. Arrows denote date of first occurrence of larval yellow perch in samples.



from South Pond reflect natural mortality rates, then alewife predation in North Pond in 1985 may have been responsible for an additional sixfold increase in the total *Z*. Although the variances in our estimates of *Z* are high, estimates for *Z* in the northern basin of North Pond are 6–60 times higher than estimates from the control site and North Pond in other years. When this result is combined with the high r^2 value for mortality rate in North Pond in 1985, a well-defined decline in larval abundance is apparent. In contrast, little of the variance is explained for larval decline in other years, and unlike in North Pond in 1985, we continued to catch larval yellow perch throughout the sampling season.

The range of values used in the predation model likely underestimated the magnitude of the impact of alewife on larval yellow perch in 1985. For example, the predation rate of individual alewife based on stomach contents may be low because alewife rapidly digest larval fish (Gannon 1976; Crowder 1980; Hunter 1981), predation may occur over a very narrow time interval, making the event difficult to observe (Brandt et al. 1987), and trawling in the vegetated region of the northern basin for alewife was ineffective at capturing alewife to estimate predation. Estimates of alewife abundance in the northern basin are not available; however, abundance estimates for alewife from other lakes range from 0.05 to 32 fish/m^2 (Lindenburg 1976; Brandt et al. 1991). Spawning aggregations of alewife in the northern basin of North Pond are expected to be more dense than lakewide averages.

Alternative sources of larval mortality

Other factors such as food availability, water temperature, and transport processes (physical and behavioral) may also contribute to estimates of larval mortality rates and could potentially be responsible for the high mortality rates estimated for North Pond in 1985. In general, the availability of sufficient concentrations of food for first-feeding larvae is important for larval survival. Prolarvae of yellow perch, however, have a complete yolk sac and larval yellow perch are somewhat resistant to starvation (Hokanson and Kleiner 1974). Moreover, in 1985, the greatest decline in larval abundance occurred within 24 h of emergence, further reducing the likelihood of starvation.

Water temperature may affect the survival of yellow perch eggs and larvae. Mean daily water temperatures in both ponds were within the limits for normal activity and survival of yellow perch larvae in 1984 and 1985 (Hokanson and Kleiner 1974). Hokanson and Kleiner considered warming rates more important for egg and early larval survival than mean water temperature. Warming rates in both ponds in 1985 (0.5°C/day) were within the range of optimal warming rates (0.5–1.0°C/day) for survival of yellow perch embryos (Hokanson and Kleiner 1974).

Embryos of yellow perch exposed to frequent temperature reversals potentially increase the probability of severe larval deformities or death (Newsome and Aalto 1987). Newsome and Aalto (1987) and Aalto and Newsome (1993) suggested that yellow perch embryos suffer from heavy losses in thermally stratified lakes because of wind-driven seiche events that expose embryos to frequent temperature reversals. North and South ponds did not undergo frequent and extreme temperature reversals in 1985 when the highest larval mortality was observed. Furthermore, temperature flucuations in South Pond were similar to those in North Pond, yet larval mortality was significantly lower. The similarities in warming rates and daily temperature fluctuations between ponds further suggest that there was little or no intrusion of the cooler Lake Ontario water. Finally, deformities were not observed when measuring larvae, and had deformities in larval body morphology been present, dramatic changes in predation mortality are unlikely because prolarvae have a poor ability to detect and escape predators (Blaxter 1986).

Another possible explanation for the larval decline observed in the northern basin of North Pond in 1985 is larval

© 1996 NRC Canada

drift into the southern basin or into Lake Ontario. Yellow perch undergo ontogenetic habitat shifts (Whiteside et al. 1985; Post and McQueen 1988; Treasurer 1988). Yellow perch spawn inshore. Soon after hatch, they move to the limnetic zone, only to return to the littoral zone as late-stage larvae and juveniles. A sufficient number of samples ($n = 2$) are not available for Lake Ontario in 1985 to directly determine if larvae were present in the lake during the period of greatest larval decline in North Pond. However, data from 1984 and 1986 suggest that larval yellow perch are retained in both the northern and southern basins of North Pond. In 1985, yellow perch were rare in the southern basin collections ($n = 52$) in contrast to 1984 and 1986 when yellow perch were relatively abundant. Storm or wind events, which may transport larvae out of the northern basin and into the southern basin of North Pond or into Lake Ontario, occurred 4 days after the period of greatest larval decline.

Yellow perch recruitment

Our study supports the hypothesis that alewife can be responsible for a substantial proportion of larval mortality and can significantly increase total larval mortality. However, can alewife affect year-class strength of yellow perch on a lakewide basis? Alewife are an exotic species in the Laurentian Great Lakes and may have caused the extinction of some native planktivorous fishes (Smith 1970; Christie 1974; Wells 1977; Crowder 1980; Eck and Wells 1987). Furthermore, alewife are an efficient planktivore and can alter the lakewide size distribution and species composition of zooplankton (Brooks and Dodson 1965; Wells 1970; Evans 1990; O'Gorman et al. 1991). It is reasonable to assume that interactions and predation intensities similar to those observed in North Pond may have occurred in other yellow perch nursery areas throughout Lake Ontario when alewife were abundant. The intensity and magnitude of such local predation events occurring throughout the lake could cause an inverse relationship between alewife abundance and year-class strength of yellow perch.

The ultimate key to understanding the overall impact of alewife on year-class development of yellow perch may be in defining the environmental cues that trigger alewife inshore migration. For example, the timing of yellow perch spawning is primarily a function of winter water temperature and duration of exposure to temperatures ≤10°C, with ambient spawning temperature a function of the prevailing temperature at the time of complete maturation (Hokanson 1977). In North Pond, the timing of yellow perch emergence was similar among years; however, alewife immigration and spawning varied by up to 2 months. The warming rates of inshore waters relative to offshore waters in Lake Ontario may be an important component driving the spring inshore migration of alewife (Collins 1973), which ultimately will determine the degree of spatial overlap between alewife and larval yellow perch.

Alewife predation can be an important source of larval yellow perch mortality. If alewife piscivory is high throughout Lake Ontario in any particular spring, the year-class strength of yellow perch could potentially be reduced. The magnitude of the lakewide reduction is likely to be determined by environmental conditions that regulate the timing of the annual inshore migrations of alewife and the degree of spatiotemporal overlap between alewife and yellow perch spawning throughout the lake.

Acknowledgments

We thank John Forney, Tim Johnson, John Kelso, Ed Mills, Mark Olson, Ed Rutherford, Craig Stow, Robert Werner, and two anonymous reviewers for commenting on various versions of the manuscript and Dave MacNeill, Carol Iancu, Andrew Davidson, Tom Coates, and Sue Proch for technical assistance. This research was funded by the National Oceanic and Atmospheric Administration's Office of Sea Grant (210-S016).

References

Aalto, S.K., and Newsome, G.E. 1993. Winds and the demic structure of a population of yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. **50**: 496–501.

Auer, N.A. 1982. Identification of larval fishes of the Great Lakes basin with emphasis on the Lake Michigan drainage. Special publication No. 82-3. Great Lakes Fishery Commission, Ann Arbor, Mich.

Blaxter, J.H.S. 1986. Development of sense organs and behavior of teleost larvae with special reference to feeding and predator avoidance. Trans. Am. Fish. Soc. **115**: 98–114.

Brandt, S.B., Mason, D.M., MacNeill, D.B., Coates, T., and Gannon, J.E. 1987. Predation by alewives on larvae of yellow perch in Lake Ontario. Trans. Am. Fish. Soc. **116**: 641–645.

Brandt, S.B., Mason, D.M., Patrick, E.V., Argyle, R.L., Wells, L., Unger, P.A., and Stewart, D.J. 1991. Acoustic measures of the abundance and size of pelagic planktivores in Lake Michigan. Can. J. Fish. Aquat. Sci. **48**: 894–908.

Brooks, J.L., and Dodson, S.I. 1965. Predation, body size and composition of plankton. Science (Washington, D.C.), **150**: 18–35.

Christie, W.J. 1974. Changes in fish species composition of the Great Lakes. J. Fish. Res. Board Can. **31**: 827–854.

Clady, M.D. 1976. The influence of temperature and wind on the survival of early stages of yellow perch, *Perca flavescens*. J. Fish. Res. Board Can. **33**: 133–138.

Collins, G.B. 1973. Factors influencing the orientation of migrating anadromous fishes. Fish. Bull. **73**: 373–398.

Crowder, L.B. 1980. Alewife, rainbow smelt, and native fishes in Lake Michigan: competition or predation? Environ. Biol. Fishes, **5**: 225–233.

Crowder, L.B., McDonald, M.E., and Rice, J.A. 1987. Understanding recruitment of Lake Michigan fishes: the importance of size-based interactions between fish and zooplankton. Can. J. Fish. Aquat. Sci. **44**(Suppl. 2): 141–147.

Doan, K.H. 1942. Some meteorological and limnological conditions as factors in the abundance of certain fishes in Lake Erie. Ecol. Monogr. **12**: 294–314.

Eck, G.W., and Wells, L. 1987. Recent changes in Lake Michigan's fish community and their probable causes, with emphasis of the role of the alewife (*Alosa pseudoharengus*). Can. J. Fish. Aquat. Sci. **44**: 53–60.

Eshenroder, R.L. 1977. Effects of intensified fishing, species changes, and spring water temperatures on yellow perch, *Perca flavescens*, in Saginaw Bay. J. Fish. Res. Board Can. **34**: 1830–1838.

Evans, M.S. 1990. Large lake responses to declines in the abundance of major fish predator: the Lake Michigan example. Can. J. Fish. Aquat. Sci. **47**: 1738–1754.

Forney, J.L. 1971. Development of dominate year classes in a yellow perch population. Trans. Am. Fish. Soc. **100**: 739–749.

Forney, J.L. 1976. Year-class formation in the walleye (*Stizostedion vitreum vitreum*) population of Oneida Lake, New York, 1966–73. J. Fish. Res. Board Can. **33**: 783–792.

Forney, J.L. 1977. Reconstruction of yellow perch (*Perca flavescens*)

© 1996 NRC Canada

cohorts from examination of walleye (*Stizostedion vitreum vitreum*) stomachs. J. Fish. Res. Board Can. **34**: 925–932.

Gannon, J.E. 1976. The effects of differential digestion rates of zooplankton by alewives, *Alosa pseudoharengus*, on determinations of selective feeding. Trans. Am. Fish. Soc. **105**: 89–95.

Hamely, J.M., Howley, T.P., and Ponhani, A.L. 1983. Estimating larval abundances from plankton net catches in Lake Point Bay, Lake Erie, in 1971–78. J. Great Lakes Res. **9**: 452–467.

Henderson, B.A. 1985. Factors affecting growth and recruitment of yellow perch, *Perca flavescens* Mitchill, in Southern Bay, Lake Huron. J. Fish Biol. **26**: 449–458.

Hile, R., and Jobes, F.W. 1940. Age, growth and production of the yellow perch, *Perca flavescens* (Mitchill), of Saginaw Bay. Trans. Am. Fish. Soc. **70**: 102–122.

Hokanson, K.E.F. 1977. Temperature requirements of some percids and adaptations to the seasonal temperature cycle. J. Fish. Res. Board Can. **34**: 1524–1550.

Hokanson, K.E.F., and Kleiner, C.F. 1974. Effects of constant and rising temperatures on survival and development rates of embryonic and larval yellow perch, *Perca flavescens* (Mitchill). *In* The early life history of fish. *Edited by* J.H.S. Blaxter. Springer-Verlag, New York. pp. 437–448.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2**: 17–29.

Hunter, J.R. 1981. Feeding ecology and predation of marine fish larvae. *In* Marine fish larvae: morphology, ecology, and relation to fisheries. *Edited by* R. Lasker. University of Washington Press, Seattle, Wash. pp. 33–77.

Jude, D.J., and Tesar, F.J. 1985. Recent changes in the inshore forage fish of Lake Michigan. Can. J. Fish. Aquat. Sci. **42**: 1154–1157.

Kohler, C.C., and Ney, J.J. 1980. Piscivority in a land-locked alewife (*Alosa pseudoharengus*) population. Can. J. Fish. Aquat. Sci. **37**: 1314–1317.

Koonce, J.F., Bagenal, T.B., Carline, R.F., Hokanson, K.E.F., and Nagiec, M. 1977. Factors influencing year-class-strength of percids: a summary and a model of temperature effects. J. Fish. Res. Board Can. **34**: 1900–1909.

Lindenburg, J.G. 1976. Seasonal depth distribution of landlocked alewives *Alosa pseudoharengus* (Wilson), in a shallow, eutrophic lake. Trans. Am. Fish. Soc. **105**: 395–399.

Mason, D.M. 1989. Influence of predation by alewives (*Alosa pseudoharengus*) on the survival of larval yellow perch (*Perca flavscens*) in a Lake Ontario embayment. M.Sc. thesis, College of Environmental Science and Forestry, State Univesity of New York, Syracuse, N.Y.

McGowen, J.A., and Brown, D.M. 1966. A new opening–closing paired zooplankton net. Reference No. 66–23. Scripps Institute of Oceanography, San Diego, Calif.

Mills, E.L., and Forney, J.L. 1981. Energetics, food consumption, and growth of young yellow perch in Oneida Lake, New York. Trans. Am. Fish. Soc. **110**: 479–488.

Newsome, G.E., and Aalto, S.K. 1987. An egg-mass census method for tracking fluctuations in yellow perch (*Perca flavescens*) populations. Can. J. Fish. Aquat. Sci. **44**: 1221–1232.

Nielsen, L.A. 1980. Effect of walleye (*Stizostedion vitreum vitreum*) predation on juvenile mortality and recruitment of yellow perch (*Perca flavescens*) in Oneida Lake, New York. Can. J. Fish. Aquat. Sci. **37**: 11–19.

O'Gorman, R., Mills, E.L., and DeGisi, J.S. 1991. Use of zooplankton to assess the movement and distribution of alewife (*Alosa pseudoharnegus*) in south-central Lake Ontario in spring. Can. J. Fish. Aquat. Sci. **48**: 2250–2257.

Post, J.R., and McQueen, D.J. 1988. Ontogenetic changes in the distribution of larval and juvenile yellow perch (*Perca flavescens*): a response to prey or predators? Can. J. Fish. Aquat. Sci. **45**: 1820–1826.

SAS Institute Inc. 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute Inc., Cary, N.C.

Smith, S.H. 1970. Species interactions of the alewife in the Great Lakes. Trans. Am. Fish. Soc. **99**: 754–765.

Storr, J.F. 1977. Lake Ontario fish tag report summary, 1972–1976. Niagara Mohawk Power Corp., Buffalo, N.Y.

Treasurer, J.W. 1988. The distribution and growth of lacustrine 0+ perch, *Perca fluviatilis*. Environ. Biol. Fishes, **21**: 37–44.

Wells, L. 1970. Effects of alewife predation on zooplankton populations in Lake Michigan. Limnol. Oceanogr. **15**: 556–565.

Wells, L. 1977. Changes in yellow perch (*Perca flavescens*) populations of Lake Michigan, 1957–75. J. Fish. Res. Board Can. **34**: 1821–1829.

Whiteside, M.C., Swindoll, C.M., and Doolittle, W.L. 1985. Factors affecting the early life history of yellow perch, *Perca flavescens*. Environ. Biol. Fishes, **12**: 47–56.

© 1996 NRC Canada

Influence of fecundity on recruitment variability of marine fish

G. Mertz and R.A. Myers

Abstract: It has often been contended that the strong variability of recruitment in marine fish populations is a consequence of their large fecundities. To test this proposal, a simple linear relationship is derived linking the standard deviation of log-recruitment for a species to its log-fecundity, and a specific prediction of the slope coefficient is provided. This result is tested using all available data for major marine fish stocks. We found that the derived relationship approximately predicts the ratio of recruitment variability to log-fecundity for the centroid of the cluster of data points, but the scatter about the centroid is so great that no significant relationship exists between recruitment variability and log-fecundity.

Résumé : On a souvent soutenu que la forte variabilité du recrutement des populations de poissons marins était une conséquence de leur grande fécondité. Pour vérifier cette hypothèse, nous avons établi une relation linéaire simple qui lie l'écart-type du logarithme du recrutement pour une espèce au logarithme de sa fécondité, et nous donnons une prédiction spécifique du coefficient de la pente. Nous testons ce résultat avec toutes les données disponibles sur les principaux stocks de poissons marins. Nous avons trouvé que la relation dérivée prédit approximativement le taux de variabilité du recrutement par rapport au logarithme de la fécondité pour le centroïde de la grappe de données ponctuelles, mais la dispersion autour du centroïde est si grande qu'il n'existe aucune relation significative entre la variabilité du recrutement et le logarithme de la fécondité.
[Traduit par la Rédaction]

Introduction

Much of fisheries science has revolved around the recruitment problem, that of discerning the factors underlying the large variations in year-class strength. It is commonly held (e.g., Houde 1987; Sale 1990; Pepin and Myers 1991; Koslow 1992) that the strong recruitment variability in marine fish is a consequence of high fecundity, or, equivalently, high total mortality from egg to recruit.

In this paper we formulate a simple equation linking recruitment variability of a species to its fecundity. This equation contains one empirical constant, relating variability of mortality to mean mortality (Bradford 1992), and it makes specific predictions of the slope of a scatter plot of the standard deviation of log-recruitment ($\sigma_{\ln R}$) versus the logarithm of fecundity. The prediction of this equation is compared with results from regressions of $\sigma_{\ln R}$ on log-fecundity, for all available marine fish species and for various taxonomic categories.

Data and methods

The data on recruitment variability were taken from the data base described in Myers et al. (1995). Briefly, Myers et al. (1995) attempted to compile all available data on spawner abundance and recruitment for marine, anadromous, and freshwater species. Spawning stock biomass and recruitment were estimated from dedicated research vessel surveys and by sequential population analysis (SPA) of commercial catch-at-age data. SPA techniques include virtual population analysis (Gulland 1965), cohort analysis (Pope 1972), and related methods that reconstruct population size from catch-at-age data (Deriso et al. 1985, 1989; Gavaris 1988; Megrey 1989). Although the focus is on populations that are strictly marine, to extend the range of fecundity we include anadromous species for which reliable data are available. (We do not examine freshwater species because our analysis relies on a regression (Bradford 1992) that did not include freshwater populations.) We only had one set of data for an elasmobranch, spiny dogfish (*Squalus acanthias*), which had a much lower fecundity than any other species examined. The estimate of recruitment for this population was based upon research vessel estimates that may overestimate the recruitment variablity (Silva 1993); we should not put too much weight on this estimate. See Myers and Barrowman (1996) for a listing of the individual stocks used in the analysis.

Two estimates of recruitment variability were examined: the standard deviation of log-transformed recruitment estimates, and the standard deviations of log residuals from a Ricker spawner recruitment function. Maximum likelihood was used to estimate the parameters of the spawner recruitment function under the assumption that the variability in recruitment for any spawner abundance was log-normally distributed (see Myers et al. (1995) for details). Similar estimates of the variability were obtained if alternative spawner recruitment functions were used.

Fecundity estimates were obtained from literature reviews, e.g., Scott and Scott (1988) and Knijn et al. (1993). We estimated the mean fecundity per year for reproductive individuals. If several batches of eggs are matured throughout the year, we attempted to estimate the sum over the year.

Formulation and analysis

The goal of this section is to obtain a testable relationship between recruitment variability and fecundity or some function of it.

The variability of a quantity is conveniently represented by its coefficient of variation. For recruitment, which tends to have a highly skewed distribution (Myers et al. 1995), it may

Received June 30, 1995. Accepted January 19, 1996.
J12977

G. Mertz[1] and R.A. Myers. Science Branch, Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, NF A1C 5X1, Canada.

[1] Author to whom all correspondence should be addressed.
e-mail: mertz@athena.nwafc.nf.ca

© 1996 NRC Canada

be appropriate to log-transform the data. The standard deviation of the log-transformed recruitment ($\sigma_{\ln R}$) may then be employed as a gauge of recruitment variability (Pepin and Myers 1991; Myers 1991). In developing the relationship between recruitment variability and variability of mortality we will briefly discuss the relationship between $\sigma_{\ln R}$ and the coefficient of variation for recruitment (CV_R).

We can write recruitment as

$$(1) \quad R(t) = E(t) \exp(-(C_1(t) + C_2(t) + \ldots + C_4(t))),$$

where t refers to a specific year, E is the total number of eggs produced, and C_i is the cumulative mortality in stage i, and for definiteness we have specified four stages (egg, early larval, late larval, and juvenile).

Let $C_i(t) = \overline{C}_i + \Delta C_i(t)$, and $\ln E(t) = \overline{\ln E} + \Delta \ln E(t)$. Then,

$$(2) \quad \ln R(t) = \overline{\ln R} + \Delta \ln E - \Sigma \Delta C_i(t),$$

where

$$(3) \quad \overline{\ln R} = \overline{\ln E} - \Sigma \overline{C}_i = \ln(E_\bullet \exp(-\Sigma \overline{C}_i)),$$

and where $E_\bullet$ is the geometric mean egg production. It is now easily shown that

$$(4) \quad \sigma_{\ln R} = (\overline{(\ln R - \overline{\ln R})^2})^{1/2} = (\overline{(\Delta \ln E - \Sigma \Delta C_i)^2})^{1/2}.$$

Note that, in general, we cannot write the mean of the squared sum as a sum of mean squares in (4) because there are likely to be correlations among some of the terms ΔE, ΔC_i (e.g., high larval survival might correspond to high juvenile mortality; Myers and Cadigan 1993*a*, 1993*b*).

If there are no correlations in mortality between stages, then (4) may be simplified to

$$(5) \quad \sigma^2_{\ln R} = \sigma^2_{\ln E} + \sigma^2_{c1} + \ldots + \sigma^2_{c4},$$

where $\sigma_{\ln E}$ is the standard deviation of log-transformed egg production and σ_{c1} is the standard deviation of mortality in stage 1, and similarly for the other stages. Relation (5) equates the variance in log-recruitment to the sum of the mortality variances plus the variance in log-transformed egg production. It is apparent from (4) and (5) that there is a very simple relationship between the recruitment variability index $\sigma_{\ln R}$ and the variability of the mortalities.

Heretofore, we have not specified the distribution for R. With the assumption that R is described by a log-normal distribution, a reasonable premise (Fogarty 1993*a*, 1993*b*), we can immediately write down, on the basis of the well-known properties of the log-normal distribution, the expression for CV_R:

$$(6) \quad CV_R = (\exp(\sigma^2_{\ln R}) - 1)^{1/2}.$$

For $\sigma_{\ln R} = 0.5$ we find from (6) that $CV_R = 0.53$ and for $\sigma_{\ln R} = 1.0$ we have $CV_R = 1.31$. Thus, it is apparent that for $CV_R \leq 1$, the two indices CV_R and $\sigma_{\ln R}$ are almost equivalent measures of recruitment variability. In the empirical treatment to follow we will find that the bulk of $\sigma_{\ln R}$ values for different fish stocks lie in the range of 0.3 to 1.0, so that there is, in general, no substantive difference between the magnitudes of these two indices. We will adopt $\sigma_{\ln R}$ as our working index, because of its more direct relation to variability of mortality ((4) and (5)).

As noted in the Data and methods section, we will work with recruitment series from which the stock (egg production) contribution to variability has been removed. Therefore, it is appropriate to neglect the $\Delta \ln E$ term in (4), and our working equation becomes

$$(7) \quad \sigma_{\ln R} = ((\Sigma \Delta C_i)^2)^{1/2}.$$

Fecundity versus variability of mortality

If the CV for total mortality prior to recruitment is close to constant across fish stocks and species, this will imply that very fecund fish will suffer higher recruitment variability. To test this posit (a constant CV for total mortality) we require an expression for total mortality that will permit its calculation from data at hand.

Toward this goal, we must introduce the equilibrium relationship between recruits and spawners. First, let $R_\bullet$ be the geometric mean recruitment, then, from (3),

$$(8) \quad E_\bullet / R_\bullet = \exp(\Sigma \overline{C}_i).$$

We assume that $R_\bullet$ can be taken to represent the equilibrium recruitment, so that

$$(9) \quad R_\bullet = ((1 - l)/\gamma) S_\bullet,$$

where $S_\bullet$ represents the equilibrium number of spawners, l is the annual survival fraction for spawners, and γ is the number of spawners per recruit. With $E_\bullet = (S_\bullet/2)F$, where F is the fecundity, then using (8) and (9), we have

$$(10) \quad \Sigma \overline{C}_i = \ln\left(\frac{\gamma}{2(1-l)} F\right) = \ln F + \ln\left(\frac{\gamma}{2(1-l)}\right).$$

This equation allows the calculation of the total mean mortality from generally available information for a fish species. The final term in (10) varies from about –0.2 to 0.7, depending on species, whereas the $\ln F$ term ranges from about 1 (sharks) to about 16 (some gadoids). For our analysis, the range of $\Sigma \overline{C}_i$ is the most important consideration and since the term $\ln(0.5\gamma/(1 - l))$ has little influence on it, it is permissible to drop this term in (10).

The hypothesis that the CV for total mortality is constant across species can be expressed as $((\Sigma \Delta C_i)^2)^{1/2}/(\Sigma \overline{C}_i) = \text{const}$, or, using (7) and (10),

$$(11) \quad \sigma_{\ln R} = \text{const} \times \ln F.$$

The constant in (11) can be obtained from simple calculations using data on the mean and variance of natural mortality compiled by Bradford (1992). The predictions of (11), with the constant specified, can be readily tested with available fisheries data.

Bradford's analysis

Bradford (1992) compiled, from the literature, daily mortality rates and their interannual variances for the egg, larval, and juvenile stages for a wide variety of marine fish. From this amalgamation of daily mortality (M) data, Bradford regressed its interannual variance (σ^2_m) on $\overline{M}$ and obtained a highly significant fit, holding across both stages and species: $\ln(\sigma^2_m) = 2.231 \ln \overline{M} - 1.893$ ($r^2 = 0.90$; $p < 0.0001$). Equivalently, we have $\sigma_m = 0.39 M^{1.12}$.

For a given stage, if we assume that $\overline{M}$ does not change from the beginning to the end of the stage, then $\overline{C} = \overline{M} \times t_s$ (t_s is the stage duration) and, correspondingly, $\sigma_c = \sigma_m \times t_s$. It follows that

© 1996 NRC Canada

Table 1. List of species used in the analysis, with fecundity per year, median standard deviation of log-recruitment, mean standard deviation of log Ricker residuals, and the number of stocks constituting the species.

	Fecundity (thousands)	Median SD	Mean Ricker SD	Number of stocks
Clupeiformes				
Clupeidae				
Brevoortia patronus (Gulf menhaden)	335.0	0.45	0.42	1
Brevoortia tyrannus (Atlantic menhaden)	335.0	0.56	0.62	1
Clupea harengus (herring)	142.0	0.80	0.74	41
Sardinops sagax (sardine)	200.0	0.71	0.68	5
Engraulidae				
Engraulis capensis (S.A. anchovy)	25.0	0.32	0.32	1
Engraulis mordax (northern anchovy)	25.0	0.83	0.84	1
Engraulis ringens (Peruvian anchoveta)	25.0	1.12	0.79	1
Gadiformes				
Gadidae				
Gadus macrocephalus (Pacific cod)	1200.0	0.86	0.88	3
Gadus morhua (cod)	2000.0	0.57	0.55	25
Melanogrammus aeglefinus (haddock)	700.0	1.06	1.08	14
Merlangius merlangus (whiting)	400.0	0.54	0.51	5
Merluccius bilinearis (silver hake)	700.0	1.05	0.71	3
Merluccius gayi (Peruvian hake)	200.0	0.24	0.18	3
Merluccius productus (Pacific hake)	79.0	1.12	1.13	1
Micromesistius poutassou (blue whiting)	200.0	0.40	0.38	2
Pollachius virens (pollock or saithe)	225.0	0.47	0.41	6
Theragra chalcogramma (walleye pollock)	600.0	0.54	0.48	5
Trisopterus esmarkii (Norway pout)	38.7	0.67	0.65	1
Urophysics tenuis (white hake)	9500.0	0.38	0.32	1
Lophiiformes				
Lophiidae				
Lophius piscatorius (monkfish)	1320.0	0.56	0.44	1
Perciformes				
Ammodytidae				
Ammodytes marinus (sandeel)	15.0	0.72	0.73	4
Carangidae				
Trachurus trachurus (horse mackerel)	364.0	0.99	0.93	2
Scombridae				
Scomber japonicus (chub mackerel)	1000.0	1.31	1.37	2
Scomber scombrus (mackerel)	400.0	0.93	0.91	2
Pleuronectiformes				
Pleuronectidae				
Eopsetta jordani (petrale sole)	800.0	0.61	0.68	1
Hippoglossoides platessoides (American plaice)	400.0	0.31	0.32	4
Hippoglossus stenolepis (Pacific halibut)	2500.0	0.37	0.36	1
Lepidopsetta bilineata (rock sole)	850.0	0.76	0.77	1
Limanda ferruginae (yellowtail flounder)	1500.0	0.82	0.65	3
Pleuronectes platessa (plaice)	80.0	0.46	0.42	8
Reinhardtius hippoglossoides (Greenland halibut)	165.0	0.15	0.15	2
Soleidae				
Solea vulgaris (sole)	350.0	0.56	0.43	7
Salmoniformes				
Osmeridae				
Mallotus villosus (capelin)	40.0	0.48	0.49	3
Salmonidae				
Oncorhynchus gorbuscha (pink salmon)	1.5	1.026	1.073	23
Oncorhynchus keta (chum salmon)	3.6	0.501	0.521	9
Oncorhynchus mykiss (steelhead trout)	4.5	0.449	0.371	3
Oncorhynchus nerka (sockeye salmon)	3.6	0.597	0.631	33
Salmo salar (Atlantic salmon)	8.3	0.320	0.345	15

© 1996 NRC Canada

Table 1 (*concluded*).

	Fecundity (thousands)	Median SD	Mean Ricker SD	Number of stocks
Scorpaeniformes				
Scorpaenidae				
Sebastes entomelas (widow rockfish)	477.5	0.83	0.81	1
Sebastes sp. (redfish)	17.5	0.66	0.55	8
Squaliformes				
Squalidae				
Squalus acanthias (spiny dogfish)	0.003	0.97	0.74	1

(12) $\sigma_c = 0.39\,\overline{M}^{\,0.12}\,\overline{C}$.

Table 1 of Bradford (1992) reveals that across stages and major fish species, $\overline{M}$ ranges typically from 0.01 to 0.1 day^{-1}, and correspondingly, the factor $\overline{M}^{\,0.12}$ ranges from 0.58 to 0.76. Clearly, little information is lost by replacing $\overline{M}^{\,0.12}$ with 0.7 and then writing $\sigma_c \approx 0.3\ \overline{C}$. Since Bradford's regression is valid across stages we can be more specific and write $\sigma_{ci} \approx 0.3\ \overline{C}_i$, where the index i indicates a specific stage. Mertz and Myers (1995) found that the slope coefficient in this equation is likely to be inflated by error in the estimation of mortality from field abundance data. Their analysis suggested that 0.2, rather than 0.3, is appropriate for the proportionality constant, and thus

(13) $\sigma_{ci} \approx 0.2\,\overline{C}_i$.

Final formulation

Equation (13) can be substituted into (5), permitting an explicit relation between $\sigma_{\ln R}$ and $\ln F$ to be derived. However, (5) is insufficiently general, since it permits no density dependence. Instead, we will apply the more general (7), after specifying a density dependence that enters through mortality in the juvenile stage. Myers and Cadigan (1993*a*) established the plausibility of density-dependent juvenile mortality for groundfish populations. In their model

(14) $\Delta C_4 = \alpha \Delta N_3 + \varepsilon$,

where ΔC_4 is the juvenile mortality minus its mean, α represents the strength of density dependence, N_3 is the abundance late in the larval stage, and ε represents the portion of juvenile mortality not associated with density dependence.

From (7) and (14) one can easily show (Mertz and Myers 1995) that

(15) $\sigma^2_{\ln R} = (1 - 2\alpha)((\sigma_{c1})^2 + (\sigma_{c2})^2 + (\sigma_{c3})^2) + (\sigma_{c4})^2$.

Myers and Cadigan (1993*a*) have shown that cod populations are subject to strong density dependence, $\alpha \approx 0.5$. This will be one limit considered here, the other being $\alpha \approx 0$.

For strong density dependence, (15) gives $\sigma_{\ln R} = \sigma_{c4}$. Using (13), we have $\sigma_{\ln R} = 0.2\,\overline{C}_4$. From Bradford's (1992) analysis, it is reasonable to treat $\overline{C}_4$ as constituting about one quarter of mean total mortality (all four stages make an appreciable contribution to total mortality). It follows that $\sigma_{\ln R} \approx 0.25 \times 0.2 \times \Sigma\,\overline{C}_i$ and thus, using (10),

(16) $\sigma_{\ln R} \approx 0.05 \ln F$

(recall that we have concluded that it is permissible to drop the final term in (10)).

For the opposite extreme of no density dependence, $\alpha = 0$, we can use (13) and (15) to write

(17) $\sigma_{\ln R} \approx 0.2\,(\overline{C}_1 2 + \ldots + \overline{C}_4 2\,)^{1/2}$.

Assuming again that there are four stages of roughly equal importance, we can write $((\overline{C}_1 2 + \ldots + \overline{C}_4 2\,)^{1/2}) \approx (1/2)((\overline{C} + \ldots + \overline{C}_4\,))$. Thus, $\sigma_{\ln R} \approx 0.1\ \Sigma\,\overline{C}_i$, and with (10) we have

(18) $\sigma_{\ln R} \approx 0.10 \ln F$.

Thus, the coefficient relating $\sigma_{\ln R}$ and $\ln F$ is about twice as large in the case of no density dependence as it is for the case of strong density dependence.

Finally, to summarize the results of this analysis, we have

(19) $\sigma_{\ln R} = (0.05 \text{ to } 0.10) \times \ln F$.

In the next section we will compare the predictions of (19) with data-based regressions.

Results

Empirical test of (19)

In Figs. 1–3 we have presented scatter plots of $\sigma_{\ln R}$ verus log-fecundity using all available marine species (Table 1) including three ovoviviparous species (in Fig. 1 only), one of which has very low fecundity, spiny dogfish. The parameters for the regressions of $\sigma_{\ln R}$ on $\ln F$ are reported in the figure captions. Note that the ordinate is shown in log to the base 10 form, while the slopes given for the linear regressions are based on $\sigma_{\ln R}$ versus the natural log of fecundity.

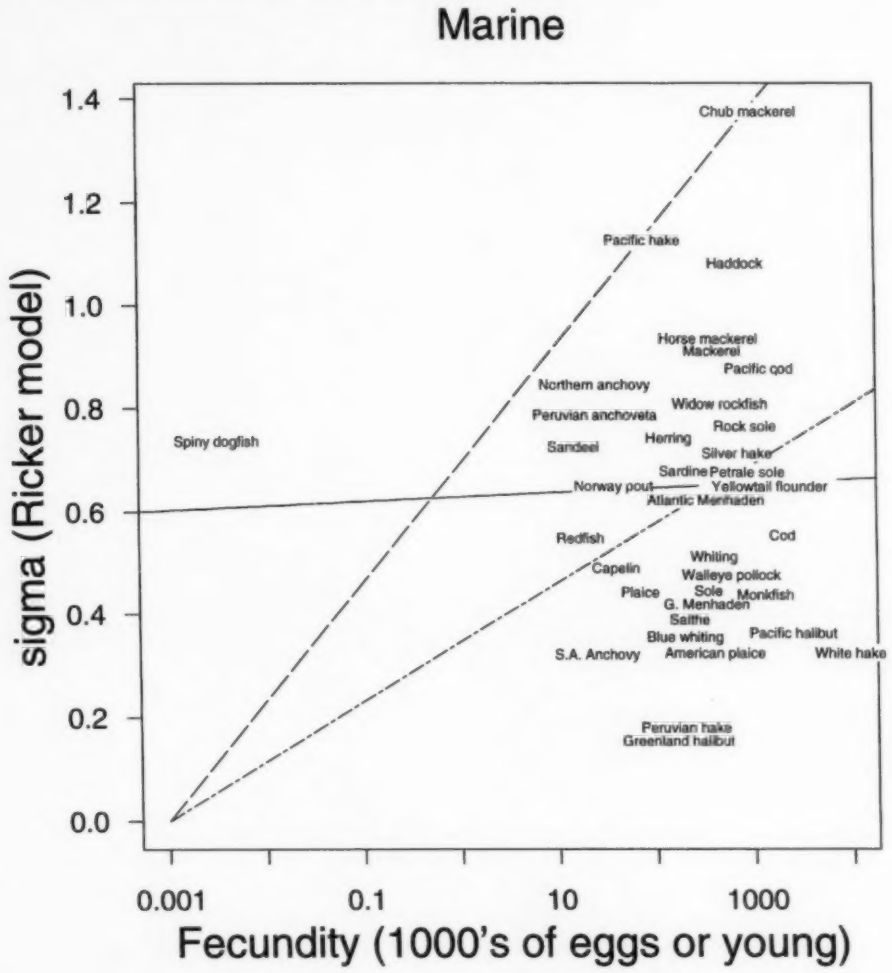
In Fig. 1 we have included (broken lines) the lines specified by the two slopes given in (19). A linear regression of $\sigma_{\ln R}$ on $\ln F$ (solid line) does not yield a significant correlation, and the resulting slope does not accord with (19).

In Fig. 2 we have removed ovoviviparous species (to eliminate spiny dogfish) and added a number of salmon species for which reliable data were available. Bradford's (1992) regression, which was used to derive the relationship between $\sigma_{\ln R}$ and $\ln F$, was based on data that included salmon populations. The inclusion of salmon species gives fairly uniform coverage over nearly four orders of magnitude in fecundity, yet there is still no indication of a significant relationship between $\sigma_{\ln R}$ and $\ln F$.

If we look at the data on the basis of orders (Fig. 3), we see that there are again no consistent relationships. In no case is the slope of $\sigma_{\ln R}$ versus $\ln F$ significantly different from zero, although for pelagic species the regression approaches statistical significance and the slope and intercept have reasonable values. We repeated the analysis eliminating stocks with less than 15 years of recruitment data; again there were no significant relationships (data not shown).

© 1996 NRC Canada

Fig. 1. Scatter plot, based on all available marine fish species, of the standard deviation of the natural log of the Ricker residuals of recruitment versus fecundity on a log (to the base 10) scale. For each species the entered label represents the mean for all available stocks constituting the species. The solid line represents the least squares fit to the data that may be expressed, in terms of ln F, as $\sigma_{\ln R} = a \ln F + b$, where $a = 0.004$, $b = 0.602$ ($r^2 = 0\%$, $p = 0.822$). The broken lines represent (19), $\sigma_{\ln R} = (0.05 \text{ to } 0.10) \ln F$.



We tested the robustness of our conclusions by considering various sources of bias in the estimates of recruitment variability. When recruitment is estimated from SPA, aging errors will cause the estimate of the variability to be negatively biased (Bradford 1991; Myers and Cadigan 1993*a*). Since aging errors nearly always increase with age (Bradford 1991), we considered only those populations in which the first age used in the SPA was less than 2, which should minimize this effect; the results still showed no relationship between recruitment variability and fecundity (Fig. 3*f*). When recruitment is estimated from research surveys, the estimate will be positively biased unless estimation methods are used to separate out the estimation error variance. When we considered the previous analyses using only research vessel series or SPA, we still could see no effect.

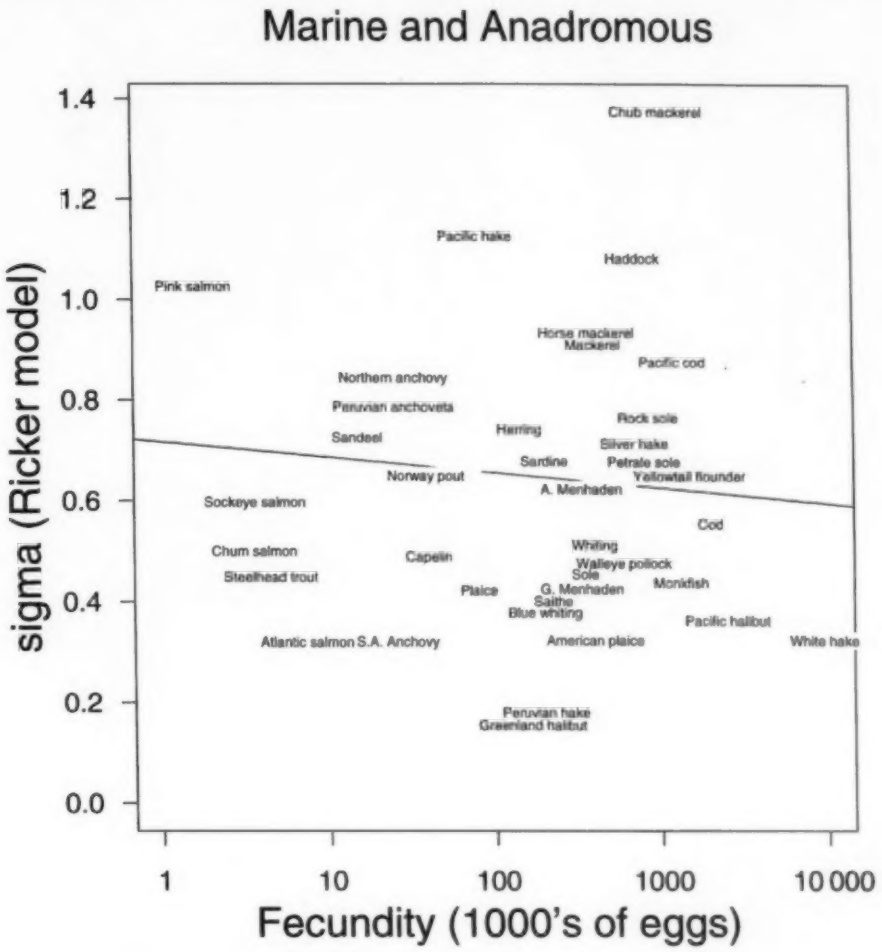
Discussion

The foregoing analysis has shown that there is no significant relationship between $\sigma_{\ln R}$ and ln F, regardless of how the data are partitioned taxonomically. (19), and its weaker form, (11), which does not specify the slope, are both rejected by the data analysis. However, the area enclosed in Fig. 1 by the limiting slopes of (19) does encompass the centroid of the cluster of data points. This suggests that (19) approximately predicts, for the centroid of the data, the correct ratio of $\sigma_{\ln R}$ to ln F. This provides some substantiation for Bradford's (1992) relation between variability of mortality and mean mortality, (12), with the correction applied by Mertz and Myers (1995).

The extreme scatter evident in Fig. 1 appears not to be consistent with the tight relationship, obtained by Bradford (1992), between variability of mortality and mean mortality.

© 1996 NRC Canada

Fig. 2. As in Fig. 1, but with the addition of salmon data (note that the ovoviviparous species have been removed), and omitting the line representing (19). For each species the entered label represents the mean for all available stocks constituting the species and the bars signify the upper and lower quintiles. The solid line (the least squares fit) is given by $\sigma_{\ln R} = 0.005 \ln F + 0.591$ ($r^2 = 0\%$, $p = 0.826$).

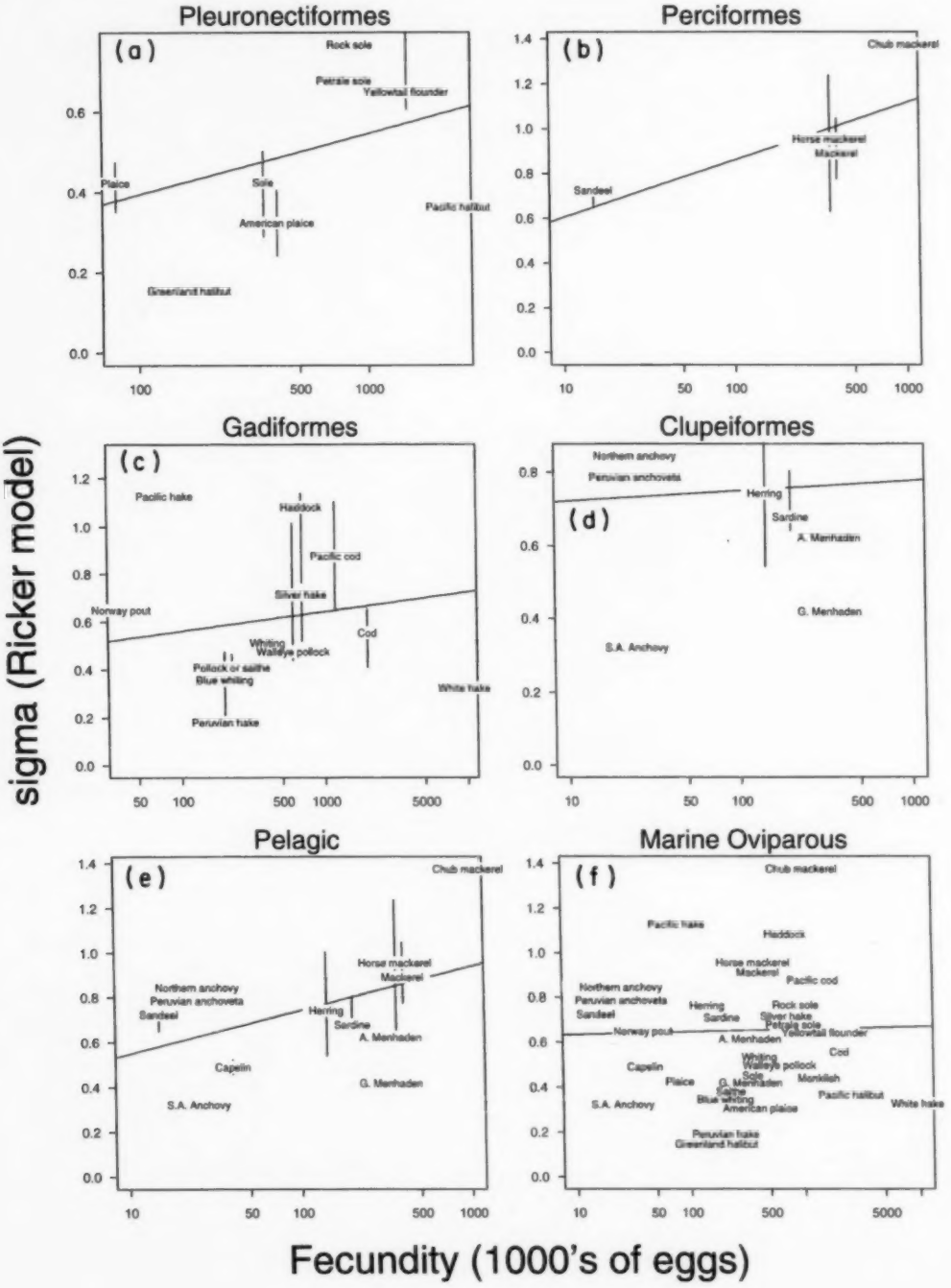


However, the gap between the two broken lines in Fig. 1 suggests that about one half the scatter can be accounted for by stock to stock differences in the strength of density dependence (α). Conceivably, the influence of density dependence could be even greater, since we somewhat arbitrarily limited α to being less than 0.5. Moreover, Bradford's relation was based on the log-transformed variances and means of mortality; obtaining a relation between recruitment variability and fecundity requires the untransformed version of the relation, (12), which is certain to have a much lower r^2. Additionally, derivation of the relationship between recruitment variability and fecundity requires summing over four stages, so that the imprecision of the variability-mean relationship for each stage becomes compounded in the final result. These factors will account, at least in part, for the scatter apparent in Fig. 1.

Our study appreciably subtracts from the plausibility of the often-proposed general relationship between variability of reproductive success and fecundity. Figures 1, 2, and 3 do not suggest a relationship between $\sigma_{\ln R}$ and $\ln F$. In particular, the data in Fig. 2 span almost four orders of magnitude in fecundity ($1.5 \times 10^3 \leq F \leq 9.5 \times 10^6$), which represents more than half the logarithmic range from very fecund fish ($F \approx 10^7$) to large (low fecundity) mammals ($F \approx 1$). The absence of a relationship over this range is forceful evidence against a general relationship between the variability of reproductive success and fecundity. Unfortunately, there are few data for species having very low fecundities, although Beddington and Basson (1994) have recently reported a stock-recruit analysis for red deer on the Isle of Rhum. They state, "It is amusing to note that the level of variation around the recruitment relationship seems to be at least in line with that observed for fish species." This lends some corroboration to our interpretation of the data.

© 1996 NRC Canada

Fig. 3. As in Fig. 2. For pleuronectiformes, $\sigma_{\ln R} = 0.066 \ln F - 0.364$ ($r^2 = 10.7\%$, $p = 0.111$). For gadiformes, $\sigma_{\ln R} = 0.035 \ln F + 0.155$ ($r^2 = 1.1\%$, $p = 0.419$). For pelagic species, $\sigma_{\ln R} = 0.085 \ln F - 0.235$ ($r^2 = 5.1\%$, $p = 0.097$). For perciformes, $\sigma_{\ln R} = 0.111 \ln F - 0.415$ ($r^2 = 24.1\%$, $p = 0.18$). For clupeiformes, $\sigma_{\ln R} = 0.012 \ln F + 0.612$ ($r^2 = 0\%$, $p = 0.90$). For marine oviparous species, those shown are those for which the first age in the sequential population analysis was less than 2 (error bars have been suppressed for graphical clarity). The regression line is $\sigma_{\ln R} = -0.005 \ln F + 0.702$ ($r^2 = 0\%$, $p = 0.857$).



© 1996 NRC Canada

Acknowledgements

M. Bradford, M. Fogarty, J. Helbig, and P. Pepin provided helpful comments on an earlier version of the manuscript. This study was supported in part by the Northern Cod Science Program of the Department of Fisheries and Oceans.

References

Beddington, J.R., and Basson, M. 1994. The limits to exploitation on land and sea. Philos. Trans. R. Soc. Lond. B Biol. Sci. **343**: 87–92.

Bradford, M.J. 1991. Effects of aging errors on recruitment time series estimated from sequential population analysis. Can. J. Fish. Aquat. Sci. **48**: 555–558.

Bradford, M.J. 1992. Precision of recruitment estimates from early life stages of marine fishes. Fish. Bull. **90**: 439–453.

Deriso, R.B., Quinn, T.J., II, and Neal, P.R. 1985. Catch-age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. **42**: 815–824.

Deriso, R.B., Neal, P.R., and Quinn, T.J., II. 1989. Further aspects of catch-age analysis with auxiliary information. *In* Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. *Edited by* R.J. Beamish and G.A. McFarlane. Can. Spec. Publ. Fish. Aquat. Sci. No. 108. pp. 127–135.

Fogarty, M.J. 1993*a*. Recruitment in randomly varying environments. ICES J. Mar. Sci. **50**: 247–260.

Fogarty, M.J. 1993*b*. Recruitment distributions revisited. Can. J. Fish. Aquat. Sci. **50**: 2723–2728.

Gavaris, S. 1988. An adaptive framework for the estimation of population size. CAFSAC research document No. 88/29. Canadian Atlantic Fisheries Centre, St. John's, Nfld.

Gulland, J.A. 1965. Estimation of mortality rates. Annex to report of the Arctic Fisheries Working Group. ICES C.M. 1965(3). International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Houde, E. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2**: 17–29.

Knijn, R.J., Boon, T.W., Heessen, H.J.L., and Hiscop, J.R. 1993. Atlas of North Sea fishes. ICES cooperative research report No. 194. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Koslow, J.A. 1992. Fecundity and the stock-recruitment relationship. Can. J. Fish. Aquat. Sci. **49**: 210–217.

Megrey, B.A. 1989. Review and comparison of age-structured stock assessment models from theoretical and applied points of view. Am. Fish. Soc. Symp. **6**: 8–48.

Mertz, G., and Myers, R.A. 1995. Estimating the predictability of recruitment. Fish. Bull. **93**: 657–665.

Myers, R.A. 1991. Recruitment variability and range of three fish species. NAFO Sci. Coun. Stud. **16**: 21–24.

Myers, R.A., and Barrowman, N.J. 1996. Is fish recruitment related to spawner abundance? Fish. Bull. **94**: 707–724.

Myers, R.A., and Cadigan, N.G. 1993*a*. Density-dependent juvenile mortality in marine demersal fish. Can. J. Fish. Aquat. Sci. **50**: 1576–1590.

Myers, R.A., and Cadigan, N.G. 1993*b*. Is juvenile natural mortality in marine demersal fish variable? Can. J. Fish. Aquat. Sci. **50**: 1591–1598.

Myers, R.A., Bridson, J., and Barrowman, N.J. 1995. Summary of worldwide spawner and recruitment data. Can. Tech. Rep. Fish. Aquat. Sci. No. 2024.

Pepin, P., and Myers, R.A. 1991. Significance of egg and larval size to recruitment variability of temperate marine fish. Can. J. Fish. Aquat. Sci. **48**: 1820–1828.

Pope, J.G. 1972. An investigation of the accuracy of virtual population analysis. ICNAF Res. Bull. No. 9. International Commission for the Northwest Atlantic Fisheries, Dartmouth, N.S. pp. 65–74.

Sale, P.F. 1990. Recruitment of marine species: is the bandwagon rolling in the right direction? Trends Ecol. Evol. **5**: 25–27.

Scott, W.B., and Scott, M.G. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. No. 219.

Silva, H.M. 1993. The causes of variability in the stock-recruitment relationship of spiny dogfish, *Squalus acanthias*, in the NW Atlantic. ICES C.M. 1993/G: 52. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

© 1996 NRC Canada

Respiratory adjustment of dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) in response to chronic turbidity

R. Brent Summers, James H. Thorp, James E. Alexander, Jr., and Ronald D. Fell

Abstract: A laboratory study was conducted to determine the effect of chronic turbidity (using bentonite clay) at medium and high temperatures on respiration of two exotic mussel species, *Dreissena polymorpha* and *Dreissena bugensis*. Populations of *D. polymorpha* from Lake Erie and the Ohio River and *D. bugensis* from Lake Erie were acclimated for 4 weeks to one of four temperature–turbidity combinations: 25°C – 0 nephelometric turbidity units (NTU), 25°C – 80 NTU, 15°C – 0 NTU, and 15°C – 80 NTU. At the end of the acclimation period, respiration was measured at both 0 and 80 NTU using a closed, flow-through system with a Clark-type polarographic microelectrode. Mass-specific respiration rates were computed as $\dot{V}O_2$ (= μL O_2 consumed·mg shell-free dry mass^{-1}·h^{-1}). Results showed that size, temperature, acclimation turbidity, and measurement turbidity significantly affected $\dot{V}O_2$ rates. An interaction between acclimation turbidity and measurement turbidity suggests that dreissenid mussels adjusted their metabolic rate in response to chronic exposure to turbidity. Mussels acclimated to higher levels (80 NTU) of turbidity did not experience as large a percent drop in $\dot{V}O_2$ when tested in turbid water (80 NTU) as did mussels acclimated at lower turbidity (0 NTU).

Résumé : Nous avons mené une étude en laboratoire pour déterminer l'effet de la turbidité chronique (à l'aide d'argile bentonitique) à des températures moyennes et élevées sur la respiration de deux espèces de mollusques exotiques, *Dreissena polymorpha* et *Dreissena bugensis*. Les populations de *D. polymorpha* du lac Érié et du fleuve Ohio et de *D. bugensis* du lac Érié ont été acclimatées pendant 4 semaines avec l'une des quatre combinaisons suivantes de température et turbidité : 25°C – 0 unités de turbidité néphélémétriques (UTN), 25°C – 80 UTN, 15°C – 0 UTN et 15°C – 80 UTN. À la fin de la période d'acclimatement, on a mesuré la respiration à 0 et à 80 UTN à l'aide d'un système fermé en circulation avec une microélectrode polarographique de type Clark. On a calculé les taux de respiration spécifiques de la masse sous la forme $\dot{V}O_2$ (= μL O_2 consommé·mg masse sèche sans coquille^{-1}·h^{-1}). Les résultats ont montré que la taille, la température, la turbidité d'acclimatement et la turbidité à la mesure avaient des effets significatifs sur les taux de $\dot{V}O_2$. Une interaction entre la turbidité d'acclimatement et la turbidité à la mesure permet de penser que les dreissenidés ont adapté leur taux métabolique en réaction à une exposition chronique à la turbidité. Les moules acclimatées à des niveaux supérieurs (80 UTN) de turbidité n'ont pas connu une baisse aussi forte en pourcentage de $\dot{V}O_2$ lorsqu'on les a testées dans une eau trouble (80 UTN) que les moules acclimatées à une turbidité plus faible (0 UTN).
[Traduit par la Rédaction]

Introduction

The spread of the zebra mussel (*Dreissena polymorpha* Pallas) and quagga mussel (*Dreissena bugensis* Andrusov) throughout eastern North America has been well documented since their initial colonization in the Great Lakes in 1986 (Hebert et al. 1989) and the former's rapid spread into the Mississippi and Ohio rivers. Zebra and quagga mussels are expected to disperse throughout much of the remainder of the central and eastern parts of the United States and the southern portion of Canada (Strayer 1991). Zebra mussels currently inhabit many large river systems east of the Rocky Mountains including the St. Lawrence, Hudson, Mississippi, Tennessee, Arkansas, and Ohio rivers. The quagga mussel is presently restricted to the lower Great Lakes and the St. Lawrence River (New York Sea Grant 1994; Mills et al. 1993); however, isolated individuals have been collected in the Mississippi River near St. Louis, Missouri. The quagga mussel is expected to spread throughout most of the range currently occupied by zebra mussels. The quagga mussel may also occupy profundal areas of lakes that are not exploited as well by zebra mussels, thus posing a new problem to native, deep water assemblages in these lentic ecosystems (Mills et al. 1993).

Dreissenid mussels in large rivers face a much different challenge in terms of both biotic and abiotic factors than do lake populations (for a review of relevant differences between lakes and large rivers, see Strayer 1991; Thorp et al. 1994). As dreissenid mussels have colonized southward from the Great Lakes into rivers, they have encountered increased turbulence, temperatures, and sediment loads (Alexander et al. 1994), as well as other differences in chemical, physical, and biological characteristics.

One important disparate factor that plagues dreissenid mussels in large river systems is the higher levels of suspended inorganic sediment (turbidity). Inorganic turbidity presents a wide range of problems for aquatic animals, particularly

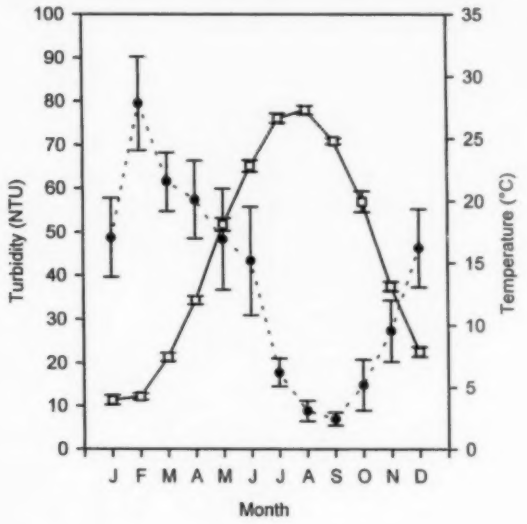
Received October 18, 1995. Accepted February 15, 1996
J13117

R.B. Summers, J.H. Thorp,[1] and J.E. Alexander, Jr. Large River Program, Biology Department, University of Louisville, Louisville, KY 40292, U.S.A.
R.D. Fell. Biology Department, University of Louisville, Louisville, KY 40292, U.S.A.

[1] Author to whom all correspondence should be addressed.
e-mail: jhthor01@ulkyvm.louisville.edu

© 1996 NRC Canada

Fig. 1. Monthly mean temperatures (open squares and solid line) and turbidity levels (solid circles and broken line) for a 14-year period (1981–1994) in the Ohio River near Louisville, Kentucky (data collected by the Louisville Water Company's Byron E. Payne water treatment plant). Error bars are standard errors of the means.



filter-feeding organisms (Wilbur 1983). The deleterious effects of inorganic turbidity on suspension-feeding bivalves include fouled gill tissues, increased pseudofecal production (Widdows et al. 1979; Bricelj and Malouf 1984), reduced feeding efficiency (Bricelj et al. 1984), and interference with respiratory surfaces (Morton 1971; Aldridge et al. 1987; McMahon 1991). Collectively, these effects impose larger costs on the energy budget of suspension-feeding bivalves. Studies by Alexander et al. (1994) examining acute effects of temperature and turbidity on *D. polymorpha* showed that respiration decreased with increasing turbidity up to 80 NTU (equivalent to 500 mg sediment/L), after which higher experimental concentrations did not have a significantly more negative effect on respiration. Respiratory rates rose with increasing temperatures ($Q_{10(acc)} = 1.96$), as is commonly seen for ectothermic animals (McMahon 1991; Alexander et al. 1994). Although riverine populations of mussels are exposed to acute bursts of inorganic turbidity as a result of short but often severe storm events (Alexander et al. 1994), they also face higher chronic levels of turbidity for a significant portion of the year in large rivers such as the Ohio (Fig. 1).

The primary goal of this study was to evaluate how chronic exposure to inorganic turbidity at medium and high temperatures affects the two species of *Dreissena* currently known present in North America. We hypothesized that the chronic effects of these two variables would differ from the acute responses, with *D. polymorpha* or *D. bugensis* adjusting their respiratory rates in response to changes in temperature and (or) turbidity. This prediction of physiological adjustment is based in part on findings of Way et al. (1990), who showed that the Asiatic clam *Corbicula fluminea* alters its physiology in response to changing environmental conditions (e.g., variable turbidity), thus maintaining optimal filtering rates. An important applied goal of this study was to contribute information that will help predict the success of these two mussels in the rivers of North America.

Materials and methods

Experimental design and maintenance

The effects of temperature and chronic exposure to suspended sediments (= turbidity) on mussel respiration were determined in a 3 × 2 × 2 experimental design. Three populations of mussels (*D. polymorpha* from Lake Erie and the Ohio River, and *D. bugensis* from Lake Erie) were obtained for the experiment. Two lentic samples of both *D. polymorpha* and *D. bugensis* were collected by scuba divers from Sandusky Bay (western Lake Erie) from 7 m depth in the fall of 1993 and summer of 1994. Samples of *D. polymorpha* were collected from the population in the Ohio River near Louisville, Kentucky, during the fall of 1993 and the spring and summer of 1994, by removing mussels from rocks in the shallow littoral zone.

Upon collection, mussels were immediately transported to the laboratory where they were sorted into size-classes of 4 mm length, placed in an environmental chamber, and acclimated to one of four combinations of temperature and turbidity under a 12 h light : 12 h dark photoperiod. Replicate samples ($N = 16$ for each treatment condition) of each population were acclimated for at least 4 weeks to one of four temperature–turbidity conditions: 15°C – 0 nephelometric turbidity units (NTU), 15°C – 80 NTU, 25°C – 0 NTU, or 25°C – 80 NTU. Temperatures in the chambers were adjusted to the acclimation level at a rate of 1°C/day, and then kept at 15 or 25°C for at least 4 weeks. Turbidity levels were maintained at 0 and 80 NTU by adding bentonite clay to U.S. Environmental Protection Agency standard freshwater (hard type; APHA 1985) in 3-L animal-holding containers that were continuously and vigorously aerated to maintain the clay in suspension. One gram per litre of bentonite clay used in this study was equal to 660 NTU. Turbidity levels were measured daily with a Hach turbidimeter (model 2100p) and adjusted as required. This 4-week temperature and turbidity acclimation period was sufficiently long to allow any physiological, morphological, or behavioral changes to take place as a function of the temperature or turbidity treatment. The temperature and turbidity levels were chosen on the basis of recent patterns of these two factors in the Ohio River. The overall inverse pattern of mean monthly temperatures and turbidity levels in the Ohio River for a 14-year period (1981–1994) is shown in Fig. 1. This presumably noncausal but significantly correlated relationship ($R^2 = -0.975$) is based on other seasonal abiotic factors such as precipitation patterns and river discharge (Alexander et al. 1994). Temperatures usually ranged from 3°C (January) to 27°C (August), while turbidities varied from 4 NTU during low flow periods to 80 NTU during high flow intervals.

While in the acclimation chamber, mussels were kept in 2-mm mesh bags within the 3-L containers and fed live *Chlorella vulgaris* every 2 days, with their water completely changed every 3 days. The number of mussels per bag depended on the size-class of mussels. For example, 20 mussels 6.0–10.0 mm long were added to a bag, whereas only 5 mussels 25.0–30.0 mm long were used. The mussels were then placed in 3.5-L plastic containers containing 3.0 L of U.S. Environmental Protection Agency standard freshwater (hard type; APHA 1985). The potentially confounding effect of body mass was controlled by using five size-classes within each treatment level for a total of 16 replicates per acclimation condition. The size-classes and replicate numbers were 6.1–10.0 mm ($N = 3$), 10.1–15.0 mm ($N = 3$), 15.1–20.0 mm ($N = 4$), 20.1–25.0 mm ($N = 3$), and 25.1–30 mm ($N = 3$).

Measurement of respiration

An experimental run consisted of four steps: (*i*) a 5-min habituation period with mussels in clear water (0 NTU), (*ii*) a 20-min measurement

© 1996 NRC Canada

Table 1. General linear model analysis of variance results.

Source of variation	df	MS	*F*	*p*
Population[a]	2	0.57	17.26	0.0001
Temperature[a]	1	5.86	176.47	0.0001
Size[a]	4	0.09	2.75	0.0001
Measurement turbidity[b]	1	26.42	982.69	0.0001
Species × measurement turbidity[b]	2	0.06	2.40	0.0944
Temperature × measurment turbidity[b]	1	0.29	10.65	0.0014
Size × measurement turbidity[b]	4	0.12	4.52	0.0018
Acclimation turbidity × measurement turbidity[b]	1	0.78	29.00	0.0001

Note: All other second and higher order interactions are nonsignificant ($\alpha = 0.05$).

[a]Error term = group (species × temperature × acclimation turbidity × size).

[b]Error term = measurement turbidity × group (species × temperature × acclimation turbidity × size).

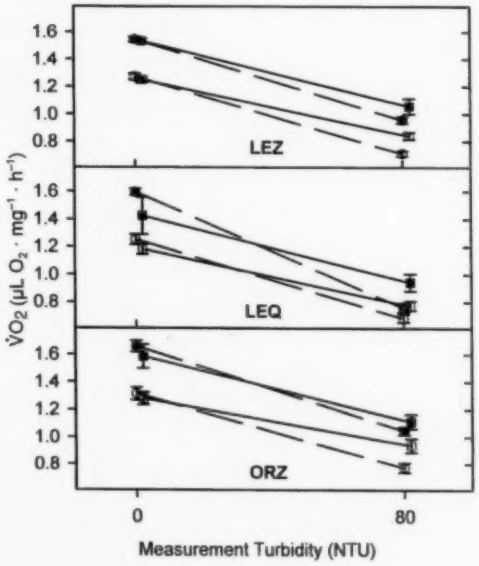
period at 0 NTU, (*iii*) a second 5-min habituation period during which the mussels were exposed to the second test turbidity of 80 NTU, and (*iv*) a second 20-min measurement period at 80 NTU. The habituation periods provided ample time for mussels to open their valves, extend their siphons, and begin filtering (cf. Alexander et al. 1994). During habituation and measurement periods, mussels were held in a cylindrical, glass respiratory chamber (9 cm long and 2.5 cm inner diameter). Holed rubber stoppers were placed at each end with stopcocks to control water flow through the clear chamber. All water was cooled or heated to the proper temperature and aerated to ensure full oxygen saturation (partial pressure of oxygen was 20.53–20.93 kPa). A perfusion pump (Gilson minipuls perfusion pump, model 312) propelled water in a complete circuit through perfusion tubes, in and out of the respiratory chamber, and past the oxygen electrode at 20 mL/min. A Cole-Parmer perfusion pump (model 7520) circulated water from a water bath through a water jacket and around a YSI oxygen microelectrode (calibrated before each series of tests) to maintain it at the proper test temperature. The continuous pumping of water through the system adequately maintained turbidity levels in the respiratory chamber. Oxygen levels were measured with a YSI oxygen meter (model 5300). The data were acquired using an analog to digital converter with a MacIntosh computer and Acknowledge (version 2.1) data acquisition software.

Shell-free, dry tissue masses were determined using the procedures outlined by McMahon (1985) and Alexander et al. (1994). After a group of animals was tested, they were dried in an oven at 85°C for several days until a constant mass was achieved. The dried tissue, which was easily removed from the shell (mantle tissue in some cases was easily removed from the inner shell wall by gently scraping), was weighed using a Cahn microbalance (model C-33). Shell-free dry biomass values were employed to calculate mass-specific oxygen consumption rates ($\dot{V}O_2$ = μL O_2 consumed·mg shell-free dry mass^{-1}·h^{-1}).

Statistical analyses

A normality test of the data showed no significant departure from a normal distribution; therefore, parametric procedures were employed for analyses using the Statistical Analysis System (SAS Institute Inc. 1990). Owing to a slightly unbalanced experimental design (unequal replicate numbers among all size-classes), a general linear models (GLM) procedure was used to perform analysis of variance. The experiment involved both a nested and split plot design (each replicate group measured at both 0 and 80 NTU). A split plot design was utilized owing to the repeated measure of respiration at both 0 and 80 NTU on the same group of animals. Main treatment effects of species, temperature, acclimation turbidity, and size were determined using the data independent of measurement turbidity, while effects relating to measurement turbidity (interactions of measurement turbidity with species, temperature, acclimation turbidity, and size) were determined separately. Expected mean squares for hypothesis tests were determined according to rules given by Damon and Harvey (1987) and used to determine appropriate error terms for tests of hypothesis (see Table 1 for mean square values and error terms). Tukey's studentized range (honestly significant difference, HSD) tests were used to perform multiple comparisons where appropriate. An alpha level of $p \leq 0.05$ was used to indicate significance of tests.

Fig. 2. Respiratory responses ($\dot{V}O_2$ = μL O_2·mg^{-1}·h^{-1}) of the zebra mussel (LEZ) and quagga mussel (LEQ) from Lake Erie, and the zebra mussel (ORZ) from the Ohio River. Mussels were acclimated for 4 weeks to one of four temperature–turbidity combinations: 25°C – 0 NTU (solid circles), 25°C – 80 NTU (solid squares), 15°C – 0 NTU (open circles), and 15°C – 80 NTU (open squares). Respiration was measured at both 0 and 80 NTU. Error bars are standard errors of the mean ($N = 16$) for each acclimation condition.



Results

Temperature and turbidity effects on respiration

Animals acclimated and measured at 25°C had significantly higher $\dot{V}O_2$ rates than those at 15°C (Fig. 2; Table 1), as is commonly seen for ectothermic animals. In general, $\dot{V}O_2$ rates dropped 25–50% (Fig. 2; Table 2) in turbid water, depending on temperature and acclimation turbidity. Mussels acclimated at 0 NTU and tested at 0 and 80 NTU showed a larger percent drop in $\dot{V}O_2$ than mussels acclimated at 80 NTU under identical test conditions (Fig. 2; Table 2). There was a significant interaction of temperature with measurement turbidity (Table 1), which indicates that mussels acclimated at 25°C had a greater depression of respiratory rate when tested under turbid conditions (80 NTU) than mussels acclimated at 15°C.

Size and species effects on respiration

Size had a significant effect on respiration (Table 1), with smaller mussels having relatively higher $\dot{V}O_2$ rates (recall that

© 1996 NRC Canada

Table 2. Percent decrease in respiratory rate ($\dot{V}O_2$) for each population or species (Lake Erie zebra mussels, LEZ; Lake Erie quagga mussels, LEQ; and Ohio river zebra mussels, ORZ) and acclimation condition (15 or 25°C; 0 or 80 NTU).

Species and acclimation temperature (°C)	Acclimation turbidity (NTU)	
	0	80
15 LEZ	43.8±0.8	32.9±1.2
25 LEZ	38.1±1.0	31.1±1.2
15 LEQ	45.2±2.2	35.3±2.5
25 LEQ	52.4±1.8	27.8±4.5
15 ORZ	39.4±4.4	25.1±4.5
25 ORZ	35.5±2.8	28.6±1.8

Note: Values represent the mean (±1 SE) percent decrease in $\dot{V}O_2$ for each species × acclimation condition measured first in clear water (0 NTU), and then in turbid water (80 NTU).

$\dot{V}O_2$ is a mass-adjusted parameter). No significant interactions occurred between size and species, size and temperature, and size and acclimation turbidity in terms of respiration (Table 1). These nonsignificant interactions suggest that respiratory rates for the three populations responded similarly among size-classes, regardless of the acclimation temperature and turbidity. In contrast, $\dot{V}O_2$ rates were influenced by a significant interaction between measurement turbidity and size (Table 1). Consequently, smaller *Dreissena* spp. had a larger percent drop in $\dot{V}O_2$ than larger mussels when they were exposed to turbid conditions immediately after testing in clear water.

Although respiration differed between populations (Table 1), the two species (including both populations of *D. polymorpha*) reacted in the same way to turbidity, temperature, acclimation turbidity, and size (Table 1). This indicates that although populations of *D. polymorpha* and *D. bugensis* had different respiratory rates, they all had the same functional response to temperature, acclimation turbidity, and size. The species difference in respiratory rate is not clear because the two populations of *D. polymorpha* (Lake Erie and Ohio River) were split into separate species–location groups for the purpose of analyzing population differences. Using a Tukey's range (HSD) multiple comparisons test, no significant differences were present between the two populations of zebra mussels (Lake Erie and Ohio River) or between Lake Erie zebra and quagga mussels. There was, however, a significant difference between Ohio River zebra mussels and Lake Erie quagga mussels. All higher order interactions relative to size or species–location group were nonsignificant ($p < 0.05$).

Acclimatory effect of turbidity

A significant interaction existed between acclimation turbidity and test turbidity (Table 1). As shown in Fig. 2, mussels acclimated at 0 NTU had higher $\dot{V}O_2$ rates than mussels acclimated at 80 NTU when tested in clear water. However, when the mussels were tested in turbid water, the mussels acclimated at 80 NTU had higher rates than those acclimated at 0 NTU. The mussels acclimated at 0 NTU showed a significantly larger percent drop in $\dot{V}O_2$ rate than did mussels acclimated at 80 NTU (Table 2). This interaction, as evident from the crossing of lines in each of the different temperature groups in Fig. 2, suggests that some form of adjustment to suspended sediment occurred during the 4-week acclimation phase of the experiment.

Discussion

Metabolic turbidity acclimation

Turbidity effects on growth, filter feeding, and to a lesser extent respiration have been widely studied in both marine and freshwater bivalve molluscs (Foster-Smith 1975; Winter 1978; Widdows et al. 1979; Kiorboe et al. 1981; Bricelj et al. 1984; Bricelj and Malouf 1984; Foe and Knight 1985; Aldridge et al. 1987; Way et al. 1990; McMahon 1991), including a few studies conducted on dreissenid mussels (Morton 1971; Sprung and Rose 1988; Reeders and Bij de Vaate 1990; Wisniewski 1990; Quigley et al. 1993; Alexander et al. 1994; Payne et al. 1995). The effects of turbidity on clams and mussels vary considerably with the sediment particle size and composition, level of turbidity, and temperatures used in the experiment (Payne et al. 1995). One effect of turbidity on bivalves is a depression of respiratory rate. In some marine and freshwater bivalves, normal levels of turbidity have moderate to relatively little effect on respiration (Widdows et al. 1979; Aldridge et al. 1987). In contrast, a study by Alexander et al. (1994) showed that respiration in the freshwater mussel *D. polymorpha* was depressed when this mollusc was exposed to relatively low levels (5 NTU) of acute turbidity, thus suggesting that dreissenid mussels may be more sensitive to suspended sediment than other tested marine and freshwater bivalve molluscs. The mechanism causing a drop in respiration with increasing turbidity is unclear. Suspended solids may overload the gut and gills (Morton 1971) or physically impede water currents or gas exchange across gill membranes (Aldridge et al. 1987; McMahon 1991).

The results of this study suggest that *D. polymorpha* and *D. bugensis* can partially acclimate to turbid water conditions by adjusting their metabolic rate. This occurs when mussels are allowed to acclimate at a moderate to high level of chronic turbidity (80 NTU bentonite clay) for an extended period (4 weeks). It appears that mussels acclimated to chronic turbidity maintain a more constant metabolic rate over a range of turbidities than mussels that have not been acclimated to turbid conditions, as evidenced by a comparison of the animals acclimated at 0 and 80 NTU within a temperature group when they were tested in turbid water (Fig. 2; Table 2). The mussels acclimated at 80 NTU did not experience as large a percent drop in $\dot{V}O_2$ as did the mussels acclimated at 0 NTU (Fig. 2; Table 2).

The factor responsible for this acclimation has not been specifically identified, but it should be either a physical, biochemical, behavioral, or combined mechanism. A behavioral change might be elicited by mussels conditioned to an environmental stimulus, such as turbidity; however, preliminary studies in our laboratory (R.B. Summers, J.H. Thorp, J.E. Alexander, Jr., and R.D. Fell, unpublished data) indicated that mussels acclimated to 80 NTU turbidity show no difference in the percent time with their valves open and filtering under different levels of turbidity (0 and 80 NTU). Biochemical changes are common for bivalves in response to a range of environmental factors (McMahon 1991). Biochemical components that may affect respiration include changes in aerobic metabolic pathways, enzyme level and activity, hemolymph and blood chemistry, substrate oxygen affinity, and gill membrane conformations (fluidity). A physical alteration may be

© 1996 NRC Canada

expressed as a modification in gill surface area, change in the structure or function of gill cilia, or a combination of factors influencing filtration and respiration rates. Physical changes such as these have been shown to occur in bivalves on both acclimatory and developmental levels. A study by Payne et al. (1995) showed that ecophenotypic differences existed within populations of both *D. polymorpha* and the Asiatic clam *Corbicula fluminea*, in which populations in habitats characterized by relatively high suspended sediments showed a marked increase in labial palp to gill area ratios. The increase in labial palp size and decrease in gill size (in *C. fluminea*) is apparently adaptive in the presence of high concentrations of suspended solids. Enlarged palp size increases the particle sorting ability of the mussels, thus allowing higher clearance and ingestion rates (Payne et al. 1995). A similar study by Way et al. (1990) demonstrated that *C. fluminea* makes physiological adjustments in response to certain environmental conditions (e.g., suspended sediment), thereby maintaining optimal filtration rates.

It is known that without time for acclimation, mussels typically reduce their clearance rate (volume of water passing through gills per unit time), thus potentially lowering their intake of oxygen and food (Aldridge et al. 1987). With a sufficient period for adjustment, mussels may acclimate to turbidity by more effectively rejecting certain particles (e.g., inorganic material). This in turn would result in higher pseudofecal production, clearance rates (without overloading the filter-feeding apparatus), and ingestion rates (B.S. Payne, Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS 39180, U.S.A., personal communication). Increases in pseudofecal production, clearance rate, and ingestion require greater energy expenditures and increased oxygen consumption. This type of acclimation appears to be the most plausible explanation of our results; however, further experimentation is necessary to determine the exact physiological basis of our findings.

The adaptation to turbidity shown here suggests that zebra and quagga mussels will be able to colonize turbid lakes and rivers at higher densities than was previously thought (see Strayer 1991). This is supported by the presence of the zebra mussel, and to a lesser extent the quagga mussel, in several river systems of North America, where substantial densities have been achieved (e.g., < 20 000 mussels/m^2 in the Ohio River). Our data indicate that the effect of turbidity on respiration, and possibly growth and reproduction, will not be as dramatic as previously reported. We predict that zebra mussel densities will not be unduly limited by the temperature range (4–30°C) and chronic turbidity levels (0–100 NTU) normally encountered in large floodplain rivers such as the Ohio and Mississippi. However, in extremely turbid rivers (chronic values < 150 NTU) mussels may be overwhelmed by the large amount of suspended and deposited sediments, thus compounding the previously mentioned problems associated with turbidity. Under these conditions of extreme chronic turbidity, dreissenid mussels may either exist at lower densities or never establish viable populations. This may be the case in an extremely turbid river such as the Missouri (Ellis et al. 1937; Pflieger and Grace 1987), which has had ample opportunities for colonization through commercial barge traffic (the primary upstream dispersal vector), but which currently lacks a viable zebra mussel population (Zebra Mussel Information Clearinghouse, Brockport, N.Y., April 1995). It is unclear if this lack of colonization can be attributed entirely to high inorganic turbidity in the Missouri River or to some other factor, such as naturally high potassium levels.

Acknowledgements

The authors thank Andy Casper, Kurt Bresko, Kim Haag, and Tim Sellers for various aspects of the research, Dr. Gary Cobbs for statistical advice, and Dr. Barry S. Payne for comments on the physiological basis of the findings. The authors also appreciate the aid of Heather Middleton in organizing Ohio River temperature and turbidity data provided by the Louisville Water Company. This research was supported by a cooperative agreement from the U.S. Environmental Protection Agency (CR820263–01–0 to J.H.T.) and a contract from the American Water Works Association Research Foundation (733–91 to J.H.T.).

References

Aldridge, D.W., Payne, B.S., and Miller, A.C. 1987. The effects of intermittent exposure to suspended solids and turbulence on three species of freshwater mussels. Environ. Pollut. **45**: 17–28.

Alexander, J.E., Thorp, J.H., and Fell, R.D. 1994. Turbidity and temperature effects on oxygen consumption in the zebra mussel (*Dreissena polymorpha*). Can. J. Fish. Aquat. Sci. **51**: 179–184.

Bricelj, V.M., and Malouf, R.E. 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. Mar. Biol. (Berlin), **84**: 155–165.

Bricelj, V.M., Malouf, R.E., and de Quillfeldt, C. 1984. Growth of juvenile *Mercenaria mercenaria* and the effect of resuspended bottom sediments. Mar. Biol. (Berlin), **84**: 167–173.

Damon, R.A., and Harvey, W.R. 1987. Experimental design, AVOVA, and regression. Harper and Row, New York.

Ellis, M.M., Westfall, B.A., and Ellis, M.D. 1937. Determination of water quality. Res. Rep. Fish Wildl. Serv. No. 9. pp. 1–122.

Foe, C., and Knight, A. 1985. The effect of phytoplankton and suspended sediment on the growth of *Corbicula fluminea* (Bivalvia). Hydrobiologia, **127**: 105–115.

Foster-Smith, R.L. 1975. The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L), and *Venerupis pullastra* (Montagu). J. Exp. Mar. Biol. Ecol. **17**: 1–22.

Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. **46**: 1587–1591.

Kiorboe, T., Mohlenberg, F., and Nohr, O. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis*. Mar. Biol. (Berlin), **61**: 283–288.

McMahon, R.F. 1985. Seasonal respiratory variation and metabolic compensation for temperature and hypoxia in the freshwater snail, *Physella virgata* (Gould), with special reference to the effects of heated effluents. Am. Malacol. Bull. **3**: 243–265.

McMahon, R.F. 1991. Mollusca: Bivalvia. Chap. 11. *In* Ecology and classification of North American freshwater invertebrates. *Edited by* J.H. Thorp and A.P. Covich. Academic Press, San Diego, Calif. pp. 315–399.

Mills, E.L., Dermott, R.M., Roseman, E.F., Dustin, D., Mellina, E., Conn, D.B., and Spidle, A.P. 1993. Colonization, ecology, and population structure of the "quagga mussel" (Bivalvia: Dreissenidae) in the lower Great Lakes. Can. J. Fish. Aquat. Sci. **50**: 2305–2314.

Morton, B.S. 1971. Studies on the biology of *Dreisesena polymorpha*

© 1996 NRC Canada

Pall. V. Some aspects of filter-feeding and the effect of micro-organisms upon the rate of filtration. Proc. Malacol. Soc. Lond. **39**: 289–301.

New York Sea Grant. 1994. North American range of the quagga mussel *Dreissena bugensis* information review. Zebra Mussel Information Clearinghouse, New York State Sea Grant, Brockport, N.Y.

Payne, B.S., Lei, J., Miller, A.C., and Hubertz, E.D. 1995. Adaptive variation in palp and gill size of the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). Can. J. Fish. Aquat. Sci. **52**: 1130–1134.

Pflieger, W.L., and Grace, T.B. 1987. Changes in the fish fauna of the lower Missouri River, 1940–1983. *In* Community and evolutionary ecology of North American stream fishes. *Edited by* D.C. Hiens and W.J. Matthews. University of Oklahoma Press, Norman, Okla. pp. 140–214.

Quigley, M.A., Gardner, W.S., and Gordon, W.M. 1993. Metabolism of the zebra mussel (*Dreissena polymorpha*) in Lake St. Clair of the Great Lakes. Chap. 18. *In* Zebra mussels: biology, impacts, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Inc., Boca Raton, Fla. pp. 295–306.

Reeders, H.H., and Bij De Vaate, A. 1990. Zebra mussels (*Dreissena polymorpha*): a new perspective for water quality management. Hydrobiologia, **200–201**: 437–450.

SAS Institute Inc. 1990. SAS/STAT users guide, version 6. 4th ed. Vols. 1–2. SAS Institute Inc., Cary, N.C.

Sprung, M., and Rose, U. 1988. Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha*. Oecologia, **77**: 526–532.

Strayer, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. Can. J. Fish. Aquat. Sci. **48**: 1389–1395.

Thorp, J.H., Alexander, J.E., Greenwood, K.S., Casper, A.F., Kessler, R.K., Black, A.R., Fang, W., Westin, A.F., Summers, R.B., Sellers, T.W., and Lewis, B.L. 1994. Predicting success of riverine populations of zebra mussels (*Dreissena polymorpha*): early colonization and microhabitat distribution in the Ohio River. *In* Proceedings of the 4th International Zebra Mussel Conference, Madison, Wis. Wisconsin Sea Grant Institute, Madison, Wis. pp. 457–476.

Way, C.M., Hornbach, D.J., Miller-Way, C.A., Payne, B.S., and Miller, A.C. 1990. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). Can. J. Zool. **68**: 115–120.

Widdows, J., Fieth, P., and Worrall, C.M. 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. (Berlin), **50**: 195–207.

Wilbur, C.G. 1983. Turbidity in the aquatic environment: an environmental factor in fresh and oceanic waters. Charles C. Thomas Publisher, Springfield, Ill.

Winter, J.E. 1978. A review of the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture, **13**: 1–33.

Wisniewski, R. 1990. Shoals of *Dreissena polymorpha* as bioprocessor of seston. Hydrobiologia, **200–201**: 451–458.

© 1996 NRC Canada

Mitochondrial DNA analysis of harbour porpoise, *Phocoena phocoena*, subpopulations in North American waters

J.Y. Wang, D.E. Gaskin, and B.N. White

Abstract: Harbour porpoises, *Phocoena phocoena*, suffer heavy incidental exploitation by commercial fisheries of North America. For effective management of this problem, information on population structure is essential. Mitochondrial DNA (mtDNA) of 204 harbour porpoises from North American waters was analysed primarily to assess population structuring and genetic diversity. Nine restriction enzymes were used to survey a mean of 588 base pairs (or 3.6%) of the estimated size of harbour porpoise mtDNA (16 346 ± 524 (SD) base pairs). Analysis of the frequency distribution of haplotypes supports the classification of some previously proposed subpopulations within the western North Atlantic and suggests that females are more philopatric than males. High diversity estimates suggest that the western North Atlantic subpopulations are not seriously depleted of genetic variation. The present study also showed that eastern North Pacific and western North Atlantic populations are fixed for haplotypes of different lineages with an estimated mean divergence of 2.3%. Under the reported rates of mtDNA evolution of 0.5–2.0%/million years, the magnitude of this divergence estimate is inconsistent with the hypothesis that divergence of eastern North Pacific and western North Atlantic harbour porpoises dates only to the Pleistocene. Recent suggestions for separate Atlantic and Pacific subspecies are also supported.

Résumé : Le marsouin commun, *Phocoena phocoena*, subit une forte exploitation dans les prises accessoires des pêches commerciales de l'Amérique du Nord. Pour aborder efficacement ce problème, il est indispensable d'avoir de l'information sur la structure de la population. Nous avons analysé l'ADN mitochondrial (ADNmt) de 204 marsouins communs des eaux de l'Amérique du Nord, pour évaluer avant tout la structure de la population et la diversité génétique. Neuf enzymes de restriction ont servi à examiner en moyenne 588 paires de bases (soit 3,6%) de la taille estimée de l'ADNmt du marsouin commun (16 346 ± 524 (ET) paires de bases). L'analyse de la distribution de fréquence des haplotypes confirme une hypothèse antérieure sur l'existence de sous-populations dans l'Atlantique Nord-Ouest, et permet de penser que les femelles sont plus philopatriques que les mâles. La forte diversité des estimations indique que la sous-population de l'Atlantique Nord-Ouest n'est pas gravement affaiblie sur le plan de la variation génétique. Notre étude a aussi montré que les populations du Pacifique Nord-Est et de l'Atlantique Nord-Ouest de différents lignages ont des haplotypes fixes, dont la divergence moyenne est estimée à 2,3%. D'après les taux signalés d'évolution de l'ADNmt, soit 0,5–2,0%/million d'années, l'ampleur de cette divergence dans les estimations ne concorde pas avec l'hypothèse selon laquelle la divergence des marsouins du Pacifique Nord-Est et de l'Atlantique Nord-Ouest daterait seulement du Pléistocène. Certaines suggestions récentes sur la séparation des sous-espèces de l'Atlantique et du Pacifique sont aussi étayées.
[Traduit par la Rédaction]

Introduction

Understanding population structuring is important for the effective management of the exploitation of any species. Analysis of mitochondrial DNA (mtDNA) has become a standard method for investigating population structure because of the rapid rate of evolution and simpler interpretation of the haploid nature of this molecule relative to nuclear DNA, and has helped define management units of many exploited species of marine mammals (e.g., Helbig et al. 1989; Stevens et al. 1989; Dizon et al. 1991; Hoelzel and Dover 1991; Schaeff et al. 1991; Wada et al. 1991; Cronin et al. 1994; Rosel et al. 1995).

In North American waters, the level of incidental capture of harbour porpoises, *Phocoena phocoena*, is of concern. The use of monofilament gill nets by commercial fisheries results in the incidental deaths of thousands of individuals each year (reviewed by Jefferson and Curry 1994). The problem in the western North Atlantic (Read and Gaskin 1988; Lien 1989; Northeast Fisheries Science Center 1992; Fontaine et al. 1994) is believed to be more serious than in the eastern North Pacific (Barlow 1987; Hofman 1990; Gearin et al. 1994; Jefferson et al. 1994; Baird and Guenther 1995). In 1990, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) classified the status of the western North Atlantic population as being threatened. Currently, the status of this population under the U.S. Endangered Species Act is being reviewed.

In the process of assessing the severity of this problem and developing effective management actions, it is necessary to understand the population structure and genetic diversity within populations of this species. Provisional divisions were

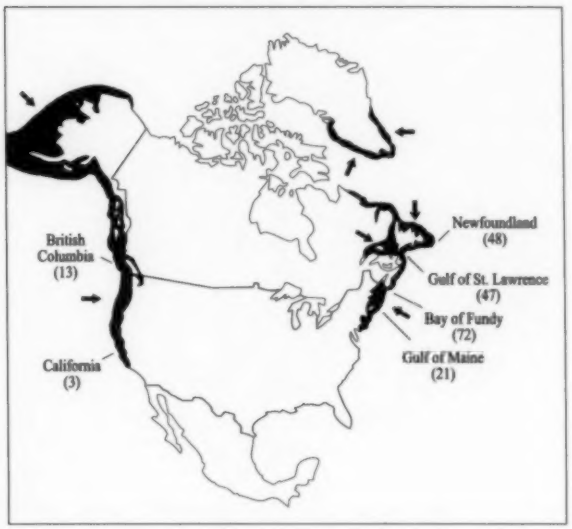
Received September 7, 1995. Accepted January 29, 1996.
J13075

J.Y. Wang[1] and D.E. Gaskin. Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.
B.N. White. Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.

[1] Author to whom correspondence should be sent at the following address: Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada. e-mail: wangjy@mcmail.mcmaster.ca

© 1996 NRC Canada

Fig. 1. Regions with consistent occurrence of harbour porpoises in North American waters (black areas). Provisional subpopulations are indicated with arrows (redrawn from Gaskin 1984). Sampled regions are also shown with sample size in parentheses.



suggested by Gaskin (1984) on the basis of differences in morphology, behaviour, geographic and oceanographic discontinuities, and migrations; these were accepted by the International Whaling Commission subcommittee on small cetaceans. The present study uses the subpopulation definitions of Gaskin (1984) and the population descriptions of Yurick and Gaskin (1987) and Amano and Miyazaki (1992), which are slightly modified from those proposed originally by Gaskin. Five populations have been described (eastern and western North Atlantic, eastern and western North Pacific, and Black Sea – Sea of Azov). Within the western North Atlantic population, Gaskin proposed five subpopulations: (*i*) west Greenland, (*ii*) southeast Greenland, (*iii*) eastern Newfoundland, (*iv*) Gulf of St. Lawrence, and (*iv*) Bay of Fundy – Gulf of Maine. In the eastern North Pacific population, he proposed two subpopulations: Bering Sea, and southern Alaska to southern California (Fig. 1). Attempts to demonstrate genetic distinctness on the basis of phenotypic characters have supported the proposed populations. In general, Atlantic porpoises have larger skulls with shorter rostrums and jaws and fewer teeth than Pacific porpoises; even smaller skulls were found in Black Sea – Sea of Azov porpoises (Tomilin 1957; Yurick 1977; Yurick and Gaskin 1987; Amano and Miyazaki 1992). However, these studies were not able to provide support for the proposed subpopulations possibly owing to limited sampling or the resolution level of the techniques. By analysing sequences of the mtDNA control region of 81 harbour porpoises from the eastern North Pacific, Rosel et al. (1995) were able to show significant heterogeneity in the distribution of haplotypes, which indicates that panmixia does not occur in this population; because of limitations in sampling, they did not test for the presence of subpopulations within the Atlantic Ocean.

In the present study, mtDNA restriction fragment length polymorphism (RFLP) analysis was performed on harbour porpoises to examine whether there is a genetic basis for the provisional subpopulations within the western North Atlantic; Greenland and Bering Sea porpoises were not available for this study. Genetic diversity was estimated to help understand the effects that exploitation may have on genetic variation in harbour porpoise populations. Eastern North Pacific porpoises were also examined to test currently accepted views of the historical zoogeography and to examine the present classification of the harbour porpoise.

Materials and methods

Tissue collection

Tissue samples from 204 harbour porpoises were obtained from five regions: Bay of Fundy (72), the Gulf of Maine (21), the Gulf of St. Lawrence (47), Newfoundland (48), and the eastern North Pacific (13 from British Columbia and 3 from California) (Fig. 1). Incidental entanglements in monofilament gill nets were the predominant source of carcasses. Tissues were preserved in a solution of 20% dimethyl sulphoxide (DMSO), 0.25 M ethylenediaminetetraacetic acid disodium salt (sodium EDTA), and sodium chloride (NaCl) (Seutin et al. 1991). Some samples were frozen (at –20°C for up to 18 months) or stored temporarily in saturated NaCl solution (for approximately 2 weeks) before being transferred into the preservative solution.

DNA extraction and restriction enzyme digestion

Total genomic DNA was extracted from approximately 0.5–1.0 g of tissue following standard phenol–chloroform procedures (details described in Wang 1993) and was digested with nine restriction enzymes following the recommendations of the manufacturer (Bethesda Research Laboratories). The enzymes had recognition sequences of 6 base pairs (bp) (*Bam*HI, *Bgl*I, *Eco*RI, and *Hin*dIII) or 4 bp (*Alu*I, *Hae*III, *Hin*fI, *Mbo*I, and *Taq*I).

Agarose gel electrophoresis and Southern transfer

DNA fragments produced by enzymes recognizing 6 bp were separated electrophoretically through 20 cm long 0.8 or 1.0% agarose gels. However, 1.5% gels were used to obtain better separation of the smaller fragments produced by digestions with enzymes recognizing 4 bp. DNA fragments in the gels were transferred onto charge-modified polyvinylidene difluoride membranes (Immobilon-N, Millipore) following Southern (1975) with slight modifications to solutions and treatment periods (gels were treated with 0.4 M NaOH, 0.6 M NaCl, and then with 0.5 M Tris-HCl, 1.5 M NaCl (pH 7.5) for 1 h each before being transferred for a minimum of 16 h). The membranes were then treated following the manufacturer's recommendations (Millipore).

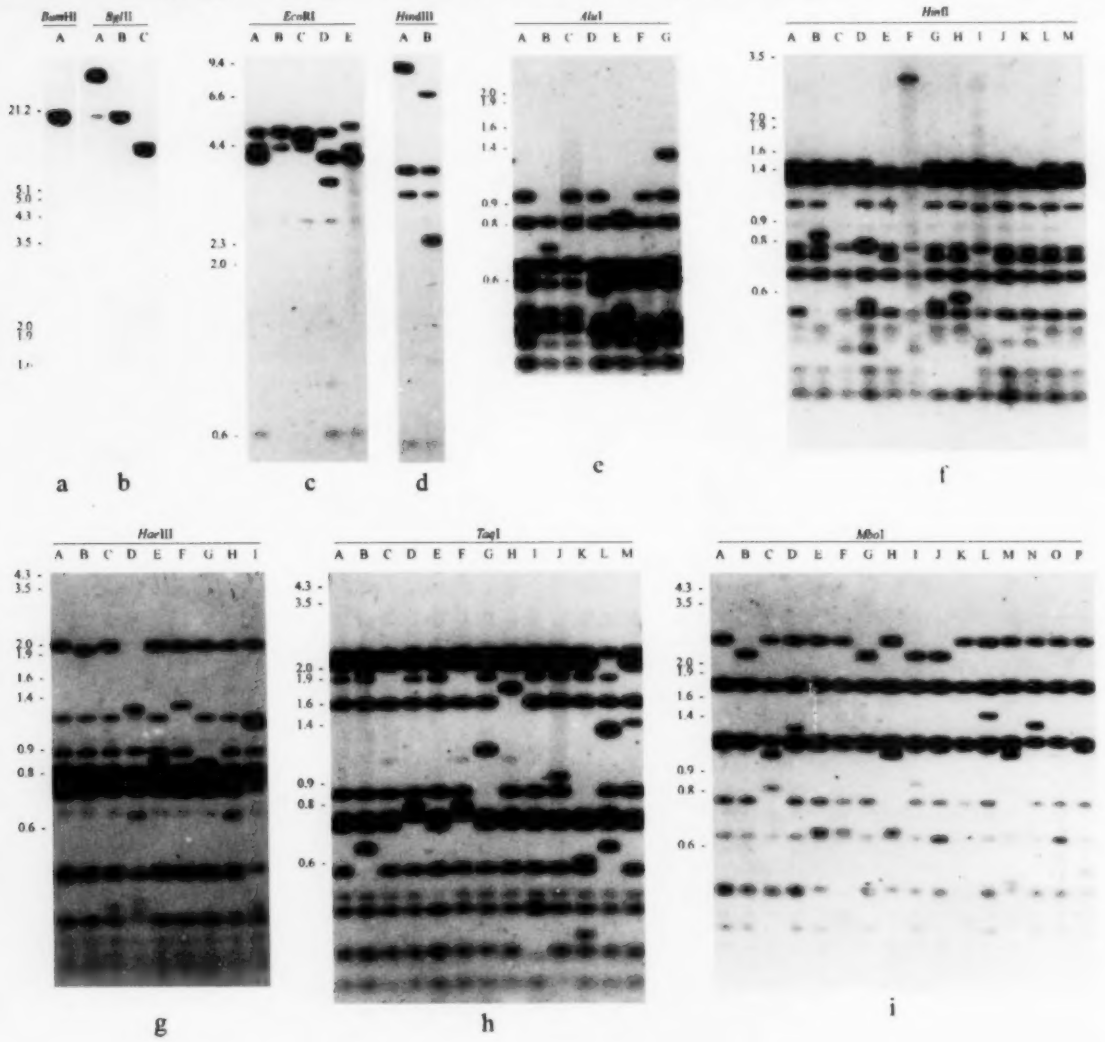
Hybridization

Each membrane was treated with 15 mL of prehybridization solution (Westneat et al. 1988) for 4–12 h at 65°C in hybridization bottles in a rotating oven before 25 ng/membrane of ^{32}P-labelled pAM1 (cloned entire mouse mtDNA, Martens and Clayton 1979) was added. Probes were radiolabelled with [α-^{32}P]dCTP (925 kBq/25 ng of probe DNA) to a specific activity of $1.0–1.5 \times 10^9$ dpm/μg by a random primer extension reaction (Feinberg and Vogelstein 1983). Hybridization with the probe lasted for 12 h. Membranes were exposed to autoradiographic film (Dupont Cronex) with a single intensifying screen (Dupont Lightning Plus) at –70°C.

For enzymes recognizing 6 bp, mtDNA restriction patterns could be identified in gels stained with ethidium bromide (Wang 1993) so Southern transfer and probing were performed only to confirm unclear restriction patterns, whenever new restriction patterns were

© 1996 NRC Canada

Fig. 2. The restriction patterns of harbour porpoise mitochondrial DNA, digested with *Bam*HI (*a*), *Bgl*II (*b*), *Eco*RI (*c*), *Hin*dIII (*d*), *Alu*I (*e*), *Hin*fI (*f*), *Hae*III (*g*), *Taq*I (*h*), and *Mbo*I (*i*). The sizes of the marker fragments, in kilobases, are indicated to the left of the restriction patterns and were produced by digesting bacteriophage lambda (*i*) DNA with *Hin*dIII (for *c* and *d*) or *Eco*RI and *Hin*dIII.



observed in stained gels or during the initial screening of samples from different collection locations.

Data organization

The molecular sizes of the restriction fragments of harbour porpoise mtDNA were estimated using standard curves of mobility versus molecular size generated by polynomial regression analysis from the mobilities of fragments of known size (computer program provided by R.G. Danzmann, University of Guelph, Guelph, Ontario, Canada). The fragments of known size were of bacteriophage lambda (λ) DNA digested with *Hin*dIII or *Eco*RI and *Hin*dIII. The smallest fragments scored varied between 280 and 390 bp depending on the restriction enzyme used (see Appendix).

A letter designation was given, in alphabetical order, to each restriction pattern (Fig. 2; Appendix) on the basis of the frequency of occurrence, starting with A for the pattern observed most commonly (except for *Alu*I patterns B and C; C occurred more frequently than B). Therefore, every porpoise could be described by a composite of nine letters representing the nine enzymes. The order of the letters in a composite corresponds to the alphabetical order of the enzyme names (i.e., *Alu*I, *Bam*HI, *Bgl*II, *Eco*RI, *Hae*III, *Hin*dIII, *Hin*fI, *Mbo*I, and *Taq*I). Each unique combination of nine letters characterized a different mtDNA haplotype.

The presence (or absence) of restriction sites was inferred from the fragment patterns with the assumption that changes in restriction sites were caused by single base substitutions within the recognition sequence (Nei and Tajima 1981). Changes in restriction sites were assessed on the basis of minimizing the number of mutational steps and homoplasy events required to produce the different fragment patterns from the most common pattern, A (see Wang 1993). Because restriction fragment information from enzymes recognizing 4 bp was incomplete (because of many fragments that were too small to detect),

© 1996 NRC Canada

many site gains or losses could not be analyzed. However, there was no reason to believe that this loss of information affected the inference of site changes or biased the estimations of pairwise divergences among the haplotypes.

Data analysis

Potential geographic, temporal, and sex-bias heterogeneity in the frequency distribution of haplotypes among harbour porpoises was tested using the χ^2 contingency test. Because of the limited sample size present for many haplotypes, the Monte Carlo bootstrapping technique of Roff and Bentzen (1989) was used to overcome the problem of small sample size. In this study, 10 000 randomizations were executed for each pairwise subpopulation comparison using the MONTE program of the restriction enzyme analysis package (REAP) version 4.1 (McElroy et al. 1992). With four provisional subpopulations, there are six pairwise comparisons possible. To ensure the level of significance for the experiment remained at 5%, the sequential Bonferroni correction technique (Rice 1989) was used to determine the critical values for each comparison. Haplotypic diversity (Nei and Tajima 1981) was estimated for each provisional subpopulation. This index of genetic variability is a function of the number and frequency of haplotypes within a sample without accounting for the relationships among haplotypes. This measure is analogous to heterozygosity estimates for nuclear DNA polymorphisms and values range from zero (monomorphism) to one (all individuals are unique).

Divergence between mtDNA haplotypes was estimated (separately for restriction enzymes recognizing 4 and 6 bp) with the program MAXLIKE (written by F. Tajima and revised by L. Jin (1988), Center for Demographic and Population Genetics, University of Texas, Houston, Texas, U.S.A.) using the maximum-likelihood restriction site approach of Nei and Tajima (1983). The average number of substitutions per nucleotide site within (nucleotide diversity (Nei 1987)) and between (nucleotide divergence (Nei and Tajima 1983)) populations was also obtained with MAXLIKE. The resulting distance matrix of pairwise divergences among haplotypes was clustered, using the neighbour-joining approach of Saitou and Nei (1987) by the NEIGHBOR algorithm of the phylogenetic inference package (PHYLIP), version 3.41 (distributed by Joe Felsenstein, Department of Genetics, University of Washington, WA 98195, U.S.A.), to produce a phenogram. Unlike the unweighted pair group method of averages (UPGMA) of Sneath and Sokal (1973), the neighbour-joining approach makes no assumptions about equal or constant rates of evolution among lineages. The phenogram was rooted to haplotype 6 of the eastern North Pacific population. An eastern North Pacific haplotype was selected as the outgroup because (*i*) eastern North Pacific harbour porpoises are believed to be isolated reproductively from the western North Atlantic animals (Gaskin 1985), and (*ii*) previous studies suggest that these populations are different morphologically (Amano and Miyazaki 1992; Yurick and Gaskin 1987) and genetically (Rosel et al. 1995).

Results

Mitochondrial DNA of 204 harbour porpoises was surveyed with each of nine enzymes. The number of different fragment patterns varied from 1 for *Bam*HI (monomorphism) to 16 for *Mbo*I (Fig. 2). A total of 129 different restriction fragments was produced with 64.1 ± 1.77 (mean ± SD) per individual (range: 58–67). From these fragments, a total of 139 different restriction sites was inferred with a mean of 105.1 ± 2.08 per individual (range: 101–110). These measurements are biased downward, since not all fragments were observed with the 4-bp enzymes. The estimated mean size of the harbour porpoise mtDNA molecule was 16 346 ± 524 bp, which is comparable to that of other cetaceans (Southern et al. 1988; Helbig et al. 1989; Stevens et al. 1989; Baker et al. 1990; Arnason et al. 1991; Dizon et al. 1991; Schaeff et al. 1991; Wada et al. 1991). This value was the mean of the sums of all of the fragments in patterns showing more than one fragment that were produced by enzymes recognizing 6 bp (i.e., *Bgl*II: pattern C, *Eco*RI and *Hin*dIII). The patterns generated by 4-bp recognition enzymes were omitted from this estimate because many fragments could not be detected consistently and confidently owing to the limited resolution of small fragments by agarose gel electrophoresis and Southern transfer (the sums of the fragments of 4-bp enzyme patterns underestimate the size of the molecule (Appendix)). An average of 588 bp was surveyed, representing approximately 3.6% of the harbour porpoise mtDNA.

Fifty-one haplotypes were identified of which 3 were found only in eastern North Pacific porpoises whereas all other haplotypes were found exclusively in the western North Atlantic population. Haplotypes 1, 2, and 3 made up 55.4% of the porpoises analyzed whereas 35 haplotypes were represented only by single individuals. Within the western North Atlantic population, 10 haplotypes were exclusive to the Bay of Fundy subpopulation, 9 to the Gulf of St. Lawrence, and 18 to eastern Newfoundland (Table 1 and Fig. 3).

Tests of heterogeneity in the distribution of mtDNA haplotypes

Since the samples were obtained opportunistically, some uncontrollable sampling biases may have arisen. However, the great mobility (Westgate et al. 1995) and loose aggregation behaviour of this species suggest that the probability of oversampling closely related individuals is low. Furthermore, very few, if any, mother–calf pairs are captured by the gill-net fisheries and no calves, as identified by unerupted teeth, were used in the present study.

Effort was made to avoid potential temporal effects on the distribution of this highly mobile species but unavoidably, sampling was carried out over two seasons in the Bay of Fundy, the Gulf of Maine, and Newfoundland regions. However, no significant temporal heterogeneity in the frequency distribution of mtDNA haplotypes within the Bay of Fundy or Newfoundland was detected by the Monte Carlo bootstrapping randomizations. Therefore, the samples collected over the two seasons were combined for regional comparisons (the Gulf of Maine samples could not be tested for temporal heterogeneity before combining because of insufficient sampling from one of the two seasons).

Managers in both Canada and the U.S.A. have been interested in whether porpoises from the Bay of Fundy and Gulf of Maine should be treated as separate management entities. Therefore, individuals from these two regions were compared prior to testing the provisional subpopulations of Gaskin (1984). No evidence for further division within the Bay of Fundy – Gulf of Maine subpopulation was found so porpoises from these two regions were not separated for the primary test of the provisional subpopulations.

Geographic heterogeneity in the frequency distribution of mtDNA haplotypes was significantly different between eastern North Pacific and all western North Atlantic regions. Within the western North Atlantic population, all subpopulations were different from each other except Gulf of St. Lawrence and Newfoundland (Table 2).

© 1996 NRC Canada

Table 1. Mitochondrial DNA haplotypes of harbour porpoises, the total number of porpoises representing each haplotype, and the frequency distribution of each haplotype in each of the five sampled regions.

			Western North Atlantic				
Haplotype	Composite	Sample size	Bay of Fundy	Gulf of Maine	Gulf of St. Lawrence	Newfoundland	Eastern North Pacific
1	AAAAAAAAA	59	26 (9)	7 (3)	15 (9)	11 (2)	—
2	AAAABAAAA	34	8 (2)	2 (1)	9 (5)	15 (8)	—
3	AAAAAABAB	20	13 (7)	6 (5)	1	—	—
4	AAAAAAADA	12	7 (7)	1 (1)	3	1	—
5	AAAAAAAEA	8	3 (2)	2 (2)	2 (1)	1	—
6	AAABDACCA	8	—	—	—	—	8 (5)
7	AABACAABA	6	2 (1)	—	4 (3)	—	—
8	BAABDACCA	4	—	—	—	—	4 (1)
9	AABACAAGA	3	1	—	1 (1)	1 (1)	—
10	CAABDAFCA	3	—	—	—	—	3 (1)
11	DABACAAJA	2	1 (1)	1	—	—	—
12	AAAAAAEFA	2	—	—	2 (1)	—	—
13	AABACAAIA	2	—	—	2 (2)	—	—
14	EAAAABAAA	2	—	1 (1)	1	—	—
15	AABABAAAA	2	—	—	—	2 (1)	—
16	AABCCADBC	2	1 (1)	1 (1)	—	—	—
17	AAAAAAAHA	1	1 (1)	—	—	—	—
18	AAAAAAAAM	1	1 (1)	—	—	—	—
19	AAAAAABAL	1	1 (1)	—	—	—	—
20	FAAAAAAIA	1	1	—	—	—	—
21	AACCCADBF	1	1 (1)	—	—	—	—
22	AAAAGAAAA	1	1	—	—	—	—
23	AABClADBH	1	1 (1)	—	—	—	—
24	GAAAAAEFA	1	1	—	—	—	—
25	AABAAAABA	1	1	—	—	—	—
26	AAAAAAMAA	1	1 (1)	—	—	—	—
27	AAAAAAANA	1	—	—	1	—	—
28	AABECAABA	1	—	—	1	—	—
29	AAAAHAADA	1	—	—	1	—	—
30	AAAABAAAJ	1	—	—	1 (1)	—	—
31	AAAAAAAMA	1	—	—	1 (1)	—	—
32	AAAAAAGOA	1	—	—	1 (1)	—	—
33	AAAAAAEKA	1	—	—	1	—	—
34	AABACADBC	1	—	—	—	1	—
35	FAAAEAAAD	1	—	—	—	1 (1)	—
36	AAAAEAJPA	1	—	—	—	1	—
37	AAAAAAAAD	1	—	—	—	1 (1)	—
38	AABAAAADA	1	—	—	—	1 (1)	—
39	AAAAFAAEG	1	—	—	—	1	—
40	AAACAAEKA	1	—	—	—	1 (1)	—
41	AAAAAAALA	1	—	—	—	1	—
42	AAAAAAKFA	1	—	—	—	1 (1)	—
43	AAADBAAAA	1	—	—	—	1	—
44	AABACALBA	1	—	—	—	1	—
45	AABACAABK	1	—	—	—	1 (1)	—
46	AACCCADBC	1	—	—	—	1	—
47	AAAAAAHHA	1	—	—	—	1 (1)	—
48	AAAAAAAAI	1	—	—	—	1	—
49	AABACAABE	1	—	—	—	1 (1)	—
50	AAAAAAADE	1	—	—	—	1	—
51	AAABDAICA	1	—	—	—	—	1
		204 (102)	72 (36)	21 (14)	47 (25)	48 (20)	16 (7)

Note: Values in parentheses represent the number of females in the sample. Letters in the composite refer to the fragment patterns (Appendix). The letters of the composite follow the order *Alu*I, *Bam*HI, *Bgl*II, *Eco*RI, *Hae*III, *Hind*III, *Hin*fI, *Mbo*I, and *Taq*I.

© 1996 NRC Canada

Table 2. Pairwise comparisons of the frequency distribution of mtDNA haplotypes of harbour porpoises (males and females combined) collected from provisional subpopulations.

	Western North Atlantic			
	Bay of Fundy – Gulf of Maine	Gulf of St. Lawrence	Newfoundland	Eastern North Pacific
Bay of Fundy – Gulf of Maine	—	0.0071±0.0000*	0.0000±0.0000*	0.0000±0.0000*
Gulf of St. Lawrence	0.0250	—	0.0701±0.0026 (ns)	0.0000±0.0000*
Newfoundland	0.0167	0.0500	—	0.0000±0.0000*
Eastern North Pacific	0.0083	0.0100	0.0125	—

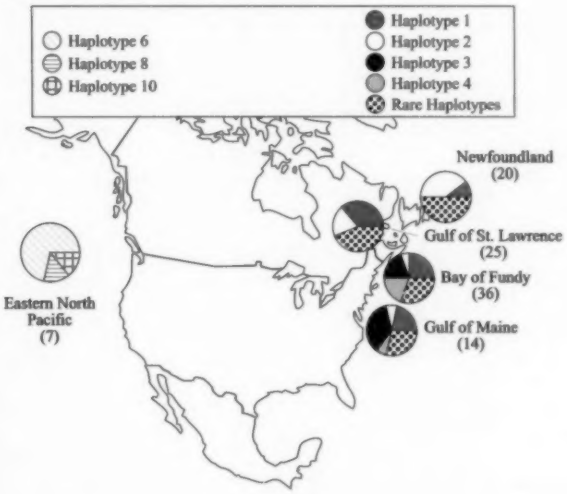
Note: The adjusted probabilities and their standard deviations were obtained following the method of Roff and Bentzen (1989). The critical probabilities for each pairwise comparison corrected with the sequential Bonferroni technique are shown below the diagonal. *, $p < 0.05$; ns, not significant.

Table 3. Pairwise comparisons of the frequency distribution of mtDNA haplotypes of female harbour porpoises collected from provisional subpopulations.

	Western North Atlantic			
	Bay of Fundy – Gulf of Maine	Gulf of St. Lawrence	Newfoundland	Eastern North Pacific
Bay of Fundy – Gulf of Maine	—	0.0007±0.0003*	0.0000±0.0000*	0.0000±0.0000*
Gulf of St. Lawrence	0.0250	—	0.0268±0.0016*	0.0001±0.0001*
Newfoundland	0.0125	0.0500	—	0.0000±0.0000*
Eastern North Pacific	0.0083	0.0167	0.0100	—

Note: The adjusted probabilities and their standard deviations were obtained following the method of Roff and Bentzen (1989). The critical probabilities for each pairwise comparison corrected with the sequential Bonferroni technique are shown below the diagonal. *, $p < 0.05$.

Fig. 3. The relative frequencies of mitochondrial DNA haplotypes found in female harbour porpoises collected from North American waters. Haplotypes are described in Table 1. Rare haplotypes consist of haplotypes 5–50 excluding haplotypes 6, 8, and 10. Sample sizes are shown in parentheses.



Observations of recognizable individual females returning annually with calves to the same general location in the Bay of Fundy (Gaskin and Watson 1985; Watson and Gaskin 1985) suggest some degree of philopatry in harbour porpoises. Moreover, philopatric behaviour has been suggested for beluga whales, *Delphinapterus leucas* (Pippard and Malcolm 1978; Pippard 1985; Caron and Smith 1990; Brennin 1992); right whales, *Eubalaena* spp. (Payne 1984; Schaeff et al. 1993); gray whales, *Eschrichtius robustus* (Swartz 1986); and humpback whales, *Megaptera novaeangliae* (Glockner and Venus 1983; Darling and Jurasz 1983; Hammond 1990; National Marine Fisheries Service 1991). Therefore, female philopatry or female population structuring in harbour porpoises was tested. Geographic heterogeneity in the frequency distribution of mtDNA haplotypes among female harbour porpoises was significant at the 5% level (Table 3) for all comparisons between provisional subpopulations. Thus, the null hypothesis of a panmictic population in the western North Atlantic was rejected. Philopatry or structuring in female harbour porpoises in the western North Atlantic was consistent with and provided genetic evidence to support the subpopulations proposed by Gaskin (1984).

Haplotypic and nucleotide diversity indices

The estimates of haplotypic diversity within harbour porpoise subpopulations varied from 0.692 (eastern North Pacific, $N = 16$) to 0.858 (Gulf of St. Lawrence, $N = 47$) and the estimates of nucleotide diversity (average number of substitutions per nucleotide site between haplotypes within populations) for these subpopulations varied from 0.194 to 0.428% (Table 4). The eastern North Pacific population had lower diversity estimates than all western North Atlantic subpopulations.

Percent site divergence between subpopulations

The calculated percent nucleotide divergence (after correcting for nucleotide diversity) varied from 0.000% (between Gulf of St. Lawrence and Newfoundland) to 1.882% (between Bay of Fundy – Gulf of Maine and eastern North Pacific) (Table 5). The estimate of the mean percent nucleotide divergence between eastern North Pacific and western North Atlantic harbour porpoises, after correction for nucleotide diversity, was approximately 1.9%.

Two major and distinct groups (lineages) of mtDNA haplotypes were resolved and can be seen in the neighbour-joining

© 1996 NRC Canada

phenogram (Fig. 4). All western North Atlantic porpoises belong to the A lineage whereas all eastern North Pacific porpoises belong to the B lineage. The percent nucleotide divergence between mtDNA haplotypes of the eastern North Pacific and western North Atlantic varied from 1.9 to 3.1% with a mean of 2.3 ± 0.27%. Within the eastern North Pacific, percent nucleotide divergence between haplotypes varied from 0.1 to 0.7% with a mean of 0.3 ± 0.21% and within the western North Atlantic, the values varied from 0.1 to 2.3% with a mean of 0.8 ± 0.44%.

Discussion

Population structure within the western North Atlantic

The present study provides genetic evidence for dividing the western North Atlantic population into smaller management units following the proposed subpopulations of Gaskin (1984) (further division of the Bay of Fundy – Gulf of Maine subpopulation was not supported). The distribution of haplotypes was significantly different among the subpopulations except for the comparison between the Gulf of St. Lawrence and Newfoundland where significance was detected only among female porpoises. However, the Monte Carlo bootstrapping analysis is conservative in detecting differences (Sokal and Rohlf 1981; R.G. Danzmann, Department of Zoology, University of Guelph, Guelph, Ontario, Canada, personal communication) and since the difference in the haplotype frequencies between these two regions was only marginally nonsignificant (i.e., the p value was very close to the critical value), further analyses with more animals or with more sensitive methods (e.g., sequencing of the mtDNA control region; analysis of variations of microsatellite DNA) are recommended.

Different results from analyses with and without male porpoises support the view that females are more philopatric than males, which is consistent with dispersal patterns of most mammals (Greenwood 1980). The lack of significant differences between the Gulf of St. Lawrence and Newfoundland when the males were included in the analysis indicates that some migration of males between the two regions may occur. Dispersal of animals over the distance between these adjacent regions for this highly vagile species is not surprising.

Genetic diversity

The estimates of haplotypic diversity for the harbour porpoise subpopulations are all comparable to those of other mammals such as the deer mice, *Peromyscus maniculatus*, of the California Channel Islands by Ashley and Wills (1987) and meadow vole, *Microtus pennsylvanicus*, by Plante et al. (1989*a*, 1989*b*). Similarly, the estimates of nucleotide diversity (average number of substitutions per nucleotide site between haplotypes within populations) for the subpopulations (range: 0.194–0.434%) are also comparable to the nucleotide diversity estimates found for other mammals such as white-tailed deer (*Odocoileus virginianus*) by Ellsworth et al. 1994; meadow vole by Plante et al. (1989*a*, 1989*b*); pocket gopher, *Geomys pinetis*, by Avise et al. (1979); and deer mice, *Peromyscus* spp., by Avise et al. (1983). However, the estimates of both haplotypic and nucleotide diversities of each of the western North Atlantic subpopulations are near the upper range for the other mammals and much greater than those of eastern North Atlantic harbour porpoises (Wang and Berggren 1995) and the humpback whale subpopulations of Baker et al. (1990) (Table 4). High nucleotide diversity for eastern North Pacific harbour porpoises was also found by Rosel et al. (1995). At least three possible biological explanations exist for the high diversity observed in the western North Atlantic population of harbour porpoises: (*i*) there is a high mutation rate in the mtDNA of harbour porpoises, (*ii*) the western North Atlantic is a large (not depleted) or expanding population, and (*iii*) there is immigration from other regions.

Table 4. Diversity indices of harbour porpoise and humpback whale mtDNA within sampled regions.

Sampled region	Sample size	Haplotypic diversity	Nucleotide diversity (×100)
Harbour porpoise			
Bay of Fundy	72	0.822	0.432
Gulf of Maine	21	0.819	0.434
Gulf of St. Lawrence	47	0.858	0.341
Newfoundland	48	0.857	0.421
Eastern North Pacific	16	0.692	0.194
Humpback whale			
Southeast Alaska	20	0.000	0.000
Hawaii	16	0.616	0.028
Central California	20	0.242	0.195
Gulf of Maine	28	0.751	0.196

Note: Haplotypic diversity was estimated using eq. 7 of Nei and Tajima (1981); values for the humpback whale were calculated from data presented in Baker et al. (1990). Nucleotide diversity within region was estimated using the maximum likelihood method (eq. 28) of Nei and Tajima (1983); values for the humpback whale are from Baker et al. (1990).

Although a fast mutation rate would produce many rare haplotypes, the presence of haplotypes that are very divergent from the three most frequently occurring types (i.e. haplotypes 1, 2, and 3) suggests that at least some haplotypes within this population are probably not the products of rapid mutation. A large or expanding population may reduce the rate of stochastic lineage extinction of mtDNA haplotypes (Wilson et al. 1985; Avise 1986) but the size and dynamics of present and historic harbour porpoise populations are currently not well known. Heavy exploitation of some eastern North Atlantic harbour porpoises (Andersen and Clausen 1984) and humpback whales (Evans 1990) has occurred and may be at least partially responsible for the much lower genetic diversity observed when compared with the western North Atlantic harbour porpoises. Immigration from other regions is a likely source of rare haplotypes because harbour porpoises are highly mobile (Gaskin et al. 1975; Read and Gaskin 1985; Westgate et al. 1995). Analysis of harbour porpoises from other regions in the North Atlantic may provide information about the level of migration between regions.

Management implications

Recognizing the Bay of Fundy – Gulf of Maine, Gulf of St. Lawrence, and Newfoundland subpopulations of Gaskin (1984) as management units was supported by the mtDNA analyses. Even though the separation of the Gulf of St. Lawrence and Newfoundland porpoises was not supported when both sexes were analysed together, we recommend the conservative approach of also recognizing them as separate

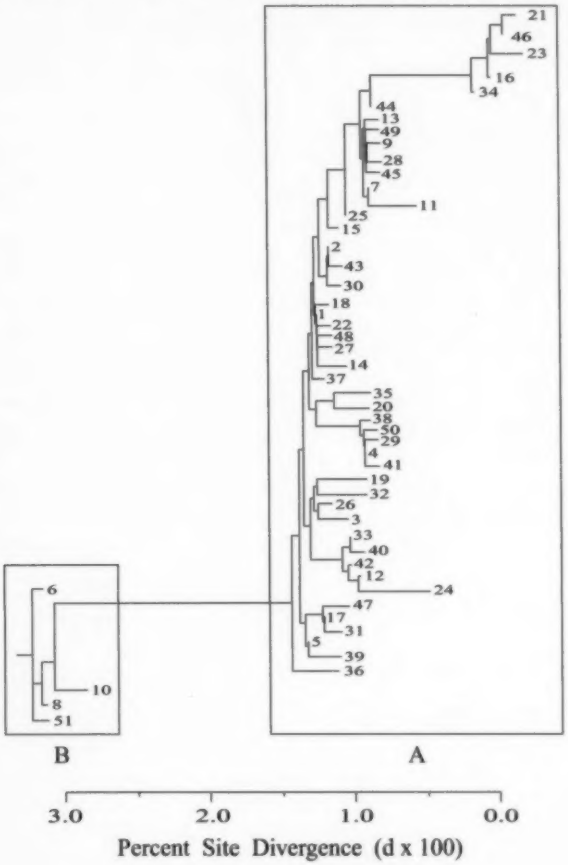
© 1996 NRC Canada

Table 5. Nucleotide divergence (× 100) estimates between provisional subpopulations of harbour porpoises corrected for nucleotide diversity within subpopulations.

	Western North Atlantic			
	Bay of Fundy – Gulf of Maine	Gulf of St. Lawrence	Newfoundland	Eastern North Pacific
Bay of Fundy – Gulf of Maine	—	0.010	0.011	1.882
Gulf of St. Lawrence	0.036	—	0.000	1.865
Newfoundland	0.044	0.041	—	1.868
Eastern North Pacific	0.033	0.033	0.036	—

Note: Standard deviations are shown below the diagonal.

Fig. 4. Neighbour-joining (Saitou and Nei 1987) phenogram of pairwise percent divergences among mitochondrial DNA haplotypes of harbour porpoises. Numbers (1 to 51) at branch termini represent the haplotypes. Box A represents the haplotypes found in the western North Atlantic Ocean and box B represents the haplotypes found in the eastern North Pacific Ocean.



management entities on the basis of the female data. Since each of these regions has high seasonal densities of harbour porpoises and high levels of incidental mortality, this approach is most prudent. In the case of the Bay of Fundy and Gulf of Maine, the frequency distributions of haplotypes between these regions were compared only in response to the interest of U.S. and Canadian managers and there were no prior biological reasons for further separating this subpopulation. Thus, we recommend the subpopulation, as described by Gaskin (1984) on the basis of nongenetic evidence, to be the management unit even though we cannot exclude the possibility that smaller divisions exist. Furthermore, diversity indices and nucleotide divergence showed essentially no difference between the Bay of Fundy and Gulf of Maine samples.

High estimates of haplotypic and nucleotide diversity for western North Atlantic subpopulations (Table 4) do not indicate serious loss of genetic variation when compared with the heavily exploited harbour porpoise subpopulations of the eastern North Atlantic (for example, haplotypic diversity (0.211) and nucleotide diversity (0.070) estimates for the Baltic Sea subpopulation (Wang and Berggren 1995) are only about 25% and 15–20%, respectively, of the same diversity estimates for the western North Atlantic subpopulations). However, since historical levels of genetic variation for these populations are not known, only by monitoring changes in the diversity indices within each subpopulation over time will we be able to assess the effects of exploitation in each region.

Historic zoogeography

Western North Atlantic and eastern North Pacific porpoises are very different as shown by the fixation of lineage A and B haplotypes, respectively. In addition, eastern North Pacific porpoises could be distinguished from western North Atlantic porpoises using the restriction patterns of any one of four enzymes (i.e., *Eco*RI pattern B (Fig. 2*c*), *Hae*III pattern D (Fig. 2*e*), *Hin*fI patterns C, F, and I (Fig. 2*h*), and *Mbo*I pattern C (Fig. 2*i*) were found only in eastern North Pacific individuals). Morphometric and meristic analyses (Yurick 1977; Yurick and Gaskin 1987; Amano and Miyazaki 1992) and recent sequence analysis of the mtDNA D-loop (Rosel et al. 1995) also indicate that reproductive isolation between these two populations is very likely.

The last opportunity for exchange to occur between the eastern North Pacific and western North Atlantic harbour porpoise populations has generally been accepted to be approximately 4000 to 13 000 years ago, during the Pleistocene (Gaskin 1976, 1985; Evans 1990; Amano and Miyazaki 1992). If these times since the isolation of these two populations are assumed, then the observed mean divergence between haplotypes of the eastern North Pacific and western North Atlantic of 2.3% would produce evolution rates of 177%/million years (for 13 000 years) to 575%/million years (for 4000 years). These unrealistic rates of evolution in such relatively recent times clearly reject any possibility that the divergence between eastern North Pacific and western North Atlantic mtDNA lineages could be so recent. However, by using the estimated rate of divergence for cetacean mtDNA of 0.5%/million years

© 1996 NRC Canada

(Hoelzel et al. 1991), the upper rate for primate mtDNA of 1.0%/million years (Foran et al. 1988), and the general mammalian mtDNA rate of 2.0%/million years (Brown et al. 1979; Wilson et al. 1985), the mean divergence between haplotypes of the eastern North Pacific and western North Atlantic harbour porpoises of 2.3% would translate into divergence times of 4.6, 2.3, and 1.2 million years ago, respectively. Rosel et al. (1995) produced a similar estimate of divergence time (5 million years). These times support the hypothesis that the initial, and crucial, isolation between mtDNA lineages of the eastern North Pacific and western North Atlantic populations occurred during the middle to late Pliocene. Furthermore, the degree of differentiation in skull morphology (Yurick 1977) suggests that this time of isolation was not limited to mtDNA lineages.

The present estimates of the mean time since haplotypes from the eastern North Pacific and western North Atlantic last shared a common ancestor reduce the importance of Pleistocene events for the dispersal of this species. Since it is improbable that both the fossil records and the dates of historic geological events, which are based on many lines of evidence, are incorrect, other explanations for the lack of congruency between the accepted views and the genetic data must first be considered. It is possible that the divergence rate for harbour porpoise mtDNA may have been underestimated by using the cetacean values of Hoelzel et al. (1991). However, even with the faster rate of other mammals, the divergence times are still much earlier than those proposed. Another possible explanation is that the mtDNA lineages A and B separated within the Pacific well before the colonization of the Atlantic. With this view, because we found fixed differences between the two populations, we must assume that either all lineage A porpoises migrated to the Atlantic or, more likely, lineage A suffered postcolonization extinction in the Pacific. It is also possible that colonization did not occur through the Canadian Arctic as believed. Since the Central American seaway was open, approximately 3.5 million years ago (Keigwin 1982), after the earliest estimated time of divergence (4.6 million years ago), it cannot be dismissed as an alternative corridor. It is impossible to deduce the actual dispersal route(s) of harbour porpoises from currently available information. Calibration and refinement of the rate of mtDNA evolution in different lineages of cetaceans and further genetic analyses of harbour porpoises from other regions are needed to test these potential dispersal routes.

Taxonomy

The most widely accepted classification within the harbour porpoise recognizes only one subspecies, *Phocoena phocoena relicta* Abel, 1905, which is a relict group restricted to the Black Sea – Sea of Azov and isolated from the North Atlantic. However, Tomilin (1957) was in favour of subspecific status for the North Pacific population, *Phocoena phocoena vomerina* (Gill, 1865), to distinguish it from the nominal North Atlantic subspecies, *Phocoena phocoena phocoena* (Linnaeus, 1758). Amano and Miyazaki (1992) also supported the subspecific status for the North Pacific population. The separation of *P. p. relicta* from Atlantic harbour porpoises is believed to have occurred as a result of the warming of the Mediterranean Sea during post-Pliocene events (Barnes 1985; Gaskin 1985). The results of the present study showed that the separation of mtDNA lineages of the eastern North Pacific and western North Atlantic predates the events leading to the isolation of *P. p. relicta*. This adds to direct evidence from comparisons of mtDNA D-loop sequences of harbour porpoises from the three oceanic basins (Rosel et al. 1995) and supports revision of the present taxonomy. If *P. p. relicta* is recognized, then subspecific status for the harbour porpoises of the North Pacific, *P. p. vomerina*, and North Atlantic, *P. p. phocoena*, should be resurrected. The other option is to remove the subspecific designation for harbour porpoises of the Black Sea – Sea of Azov.

In conclusion, structuring within the western North Atlantic population of harbour porpoises was detected and our data clearly showed the differences between the western North Atlantic and eastern North Pacific populations. The genetic evidence supported many proposed subpopulations within the western North Atlantic. Furthermore, the high mtDNA diversity of all three subpopulations within the western North Atlantic indicates that this population probably has not experienced a severe bottleneck event in its recent history. Finally, this study also provided evidence to suggest that the currently accepted views of both historic zoogeography and taxonomy of this species require further examination.

Acknowledgements

We thank R. Baird, A. Evely, P. Fontaine, J. Harvey, M. Kingsley, P. Lieberg-Clark, J. Nicolas, H. Rhinehart, S. Richardson, G. Stenson, and the researchers at the Grand Manan Whale and Seabird Research Station for providing samples from their regions. We also thank T. Crease, R. Danzmann, L. Dueck, J. Marsolais, and A. Neimanis for their assistance in the analyses. Furthermore, we thank P. Rosel, J. Clayton, and an anonymous reviewer for constructive criticism that greatly improved earlier drafts of the manuscript. This study was supported by operating grants to B.N.W. and D.E.G. from the Natural Sciences and Engineering Research Council of Canada.

References

Amano, M., and Miyazaki, N. 1992. Geographic variation in skulls of the harbour porpoise, *Phocoena phocoena*. Mammalia, **56**: 133–144.

Andersen, S., and Clausen, B. 1984. Bycatches of the harbour porpoise, *Phocoena phocoena* in Danish fisheries 1980–1981, and evidence for overexploitation. Rep. Int. Whal. Comm. **34**: 745. (Abstr.)

Arnason, U., Gullberg, A., and Widegren, B. 1991. The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus*. J. Mol. Evol. **33**: 556–568.

Ashley, M., and Wills, C. 1987. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. Evolution, **41**: 854–863.

Avise, J.C. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Philos. Trans. R. Soc. Lond. B Biol. Sci. **312**: 325–342.

Avise, J.C., Giblin-Davidson, C., Laerm, J., Patton, J.C., and Lansman, R.A. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proc. Natl. Acad. Sci. U.S.A. **76**: 6694–6698.

Avise, J.C., Shapira, J.F., Daniel, S.A., Aquadro, C.F., and Lansman, R.A. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. **1**: 38–56.

© 1996 NRC Canada

Baird, R.W., and Guenther, T.J. 1995. Account of harbour porpoise (*Phocoena phocoena*) strandings and bycatches along the coast of British Columbia. Rep. Int. Whal. Comm. (Spec. Issue) No. 16. pp. 159–168.

Baker, C.S., Palumbi, S.R., Lambertsen, R.H., Weinrich, M.T., Calambokidis, J., and O'Brien, S.J. 1990. Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. Nature (London), **344**: 238–240.

Barlow, J. 1987. An assessment of the status of harbor porpoise populations in central California. Administrative report No. LJ–87–06. National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, Calif.

Barnes, L.G. 1985. Evolution, taxonomy and antitropical distributions of the porpoises (Phocoenidae, Mammalia). Mar. Mamm. Sci. **1**: 149–165.

Brennin, R. 1992. Population genetic structure of beluga whales, *Delphinapterus leucas*, mitochondrial DNA sequence variation within and among North American populations. M.Sc. thesis, McMaster University, Hamilton, Ont.

Brown, W.M., George, M., Jr., and Wilson, A.C. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. **76**: 1967–1971.

Caron, L.M.J., and Smith, T.G. 1990. Philopatry and site tenacity of belugas, *Delphinapterus leucas*, hunted by the Inuit at the Nastapoka estuary, eastern Hudson Bay. *In* Advances in research on the beluga whale, *Delphinapterus leucas*. *Edited by* T.G. Smith, D.J. St. Aubin, and J.R. Geraci. Can. Bull. Fish. Aquat. Sci. No. 224. pp. 69–79.

Cronin, M.A., Hills, S., Born, E.W., and Patton, J.C. 1994. Mitochondrial DNA variation in Atlantic and Pacific walruses. Can. J. Zool. **72**: 1035–1043.

Darling, J.D., and Jurasz, C.M. 1983. Migratory destination of North Pacific humpback whales (*Megaptera novaeangliae*). *In* Communication and behavior of whales. AAAS selected symposia series. *Edited by* R. Payne. Westview Press, Boulder, Colo. pp. 359–368.

Dizon, A.E., Southern, S.O., and Perrin, W.F. 1991. Molecular analysis of mtDNA types in exploited populations of spinner dolphins (*Stenella longirostris*). Rep. Int. Whal. Comm. (Spec. Issue) No. 13. pp. 183–202.

Ellsworth, D.L., Honeycutt, R.L., Silvy, N.J., Bickham, J.W., and Klimstra, W.D. 1994. Historical biogeography and contemporary patterns of mitochondrial DNA variation in white-tailed deer from the southeastern United States. Evolution, **48**: 122–136.

Evans, P.G.H. 1990. The natural history of whales and dolphins. Facts On File Publications, New York.

Feinberg, A.P., and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 6–13.

Fontaine, P.M., Barrette, C., Hammill, M.O., and Kingsley, M.C.S. 1994. Incidental catches of harbour porpoise (*Phocoena phocoena*) in the Gulf of St. Lawrence and the St. Lawrence River Estuary, Quebec, Canada. Rep. Int. Whal. Comm. (Spec. Issue) No. 15. pp. 159–164.

Foran, D.R., Hixson, J.E., and Brown, W.M. 1988. Comparisons of ape and human sequences that regulate mitochondrial DNA transcription and D-loop DNA synthesis. Nucleic Acids Res. **16**: 5841–5861.

Gaskin, D.E. 1976. The evolution, zoogeography and ecology of Cetacea. Oceanogr. Mar. Biol. Annu. Rev. **14**: 247–346.

Gaskin, D.E. 1984. The harbour porpoise *Phocoena phocoena* (L.): regional populations, status, and information on direct and indirect catches. Rep. Int. Whal. Comm. **34**: 569–586.

Gaskin, D.E. 1985. The ecology of whales and dolphins. Heinemann, London.

Gaskin, D.E., and Watson, A.P. 1985. The harbour porpoise, *Phocoena phocoena*, in Fish Harbour, New Brunswick, Canada: occupancy, distribution, and movements. Fish. Bull. **83**: 427–442.

Gaskin, D.E., Smith, G.J.D., and Watson, A.P. 1975. Preliminary study of harbour porpoises (*Phocoena phocoena*) in the Bay of Fundy using radiotelemetry. Can. J. Zool. **53**: 1466–1471.

Gearin, P.J., Melin, S.R., DeLong, R.L., and Kajimura, H. 1994. Harbor porpoise interactions with a chinook salmon set-net fishery in Washington state. Rep. Int. Whal. Comm. (Spec. Issue) No. 15. pp. 427–438.

Glockner, D.A., and Venus, S.C. 1983. Identification, growth rate, and behavior of humpback whale (*Megaptera novaeangliae*) cows and calves in the waters off Maui, Hawaii, 1977–79. *In* Communication and behavior of whales. AAAS selected symposia series. *Edited by* R. Payne. Westview Press, Boulder, Colo. pp. 223–258.

Greenwood, P.J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. Anim. Behav. **28**: 1140–1162.

Hammond, P.S. 1990. Capturing whales on film: estimating cetacean population parameters from individual recognition data. Mammal Rev. **20**: 17–22.

Helbig, R., Boag, P.T., and White, B.N. 1989. Stock identification of beluga whales (*Delphinapterus leucas*) using mitochondrial DNA markers: preliminary results. Musk-ox, **37**: 122–128.

Hoelzel, A.R., and Dover, G.A. 1991. Genetic differentiation between sympatric killer whale populations. Heredity, **66**: 191–195.

Hoelzel, A.R., Hancock, J.M., and Dover, G.A. 1991. Evolution of the cetacean mitochondrial D-loop region. Mol. Biol. Evol. **8**: 475–493.

Hofman, R.J. 1990. Cetacean entanglement in fishing gear. Mammal Rev. **20**: 53–64.

Jefferson, T.A., and Curry, B.E. 1994. A global review of porpoise (Cetacea: Phocoenidae) mortality in gillnets. Biol. Conserv. **67**: 167–183.

Jefferson, T.A., Curry, B.E., and Black, N.E. 1994. Harbour porpoise mortality in the Monterey Bay halibut gillnet fishery, 1989. Rep. Int. Whal. Comm. (Spec. Issue) No. 15. pp. 445–448.

Keigwin, L. 1982. Isotopic palaeoceanography of the Caribbean and East Pacific: role of Panama uplift in late Neogene time. Science (Washington, D.C.), **217**: 350–353.

Lien, J. 1989. Incidental catch of harbour porpoise (*Phocoena phocoena*) in waters off Newfoundland and Labrador: some estimates based on present data and a request for further study. Report to the workshop on harbour porpoise, 22 August 1989. St. Andrews Biological Station, Department of Fisheries and Oceans, St. Andrews, N.B.

Martens, P.A., and Clayton, D.A. 1979. Mechanism of mitochondrial DNA replication in mouse L-cells: localization and sequence of the light-strand origin of replication. J. Mol. Biol. **135**: 327–351.

McElroy, D., Morgan, P., Bermingham, E., and Kornfield, I. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. **83**: 157–158.

National Marine Fisheries Service. 1991. Recovery plan for the humpback whale (*Megaptera novaeangliae*). Prepared by the humpback whale recovery team for the National Marine Fisheries Service, Silver Spring, Md.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics, **97**: 145–163.

Nei, M., and Tajima, F. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics, **105**: 207–217.

Northeast Fisheries Science Center. 1992. Harbor porpoise in eastern North America: status and research needs (result of a scientific workshop held May 5–8, 1992). Reference document No. 92–06. Northeast Fisheries Science Center, Woods Hole, Mass.

Payne, R. 1984. Reproductive rates and breeding area occupancy in the southern right whale, *Eubalaena australis*. Rep. Int. Whal. Comm. (Spec. Issue) No. 6. p. 482. (Abstr.)

© 1996 NRC Canada

Pippard, L. 1985. Status of the St. Lawrence River population of beluga, *Delphinapterus leucas*. Can. Field-Nat. **99**: 438–450.

Pippard, L., and Malcolm, H. 1978. White whales (*Delphinapterus leucas*): observations on their critical habitats in the St. Lawrence and Saguenay rivers. Department of Indian and Northern Affairs, Parks Canada, Ottawa, Ont.

Plante, Y., Boag, P.T., and White, B.N. 1989*a*. Microgeographic variation in mitochondrial DNA of meadow voles (*Microtus pennsylvanicus*) in relation to population density. Evolution, **43**: 1522–1537.

Plante, Y., Boag, P.T., and White, B.N. 1989*b*. Macrogeographic variation in mitochondrial DNA of meadow voles (*Microtus pennsylvanicus*). Can. J. Zool. **67**: 158–167.

Read, A.J., and Gaskin, D.E. 1985. Radio tracking the movements and activities of harbor porpoises, *Phocoena phocoena* (L.), in the Bay of Fundy, Canada. Fish. Bull. **83**: 543–552.

Read, A.J., and Gaskin, D.E. 1988. Incidental catch of harbour porpoises by gill nets. J. Wildl. Manage. **52**: 517–523.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Roff, D.A., and Bentzen, P. 1989. The statistical analysis of mtDNA polymorphisms: chi-square and the problem of small samples. Mol. Biol. Evol. **6**: 539–545.

Rosel, P.E., Dizon, A.E., and Haygood, M.G. 1995. Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. Can. J. Fish. Aquat. Sci. **52**: 1210–1219.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Schaeff, C.M., Kraus, S.D., Brown, M., Perkins, J., Payne, R., Gaskin, D., Boag, P., and White, B. 1991. Preliminary analysis of mitochondrial DNA variation within and between right whale species *Eubalaena glacialis* and *Eubalaena australis*. Rep. Int. Whal. Comm. (Spec. Issue) No. 13. pp. 217–223.

Schaeff, C.M., Kraus, S.D., Brown, M.W., and White, B.N. 1993. Assessment of the population structure of western North Atlantic right whales (*Eubalaena glacialis*) based on sighting and mtDNA data. Can. J. Zool. **71**: 339–345.

Seutin, G., White, B.N., and Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. **69**: 82–90.

Sneath, P.H.A., and Sokal, R.R. 1973. Numerical taxonomy. W.H. Freeman and Co., San Francisco, Calif.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. 2nd ed. W.H. Freeman and Co., San Francisco, Calif.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.

Southern, S.O., Southern, P.J., and Dizon, A.E. 1988. Molecular characterisation of a cloned dolphin mitochondrial genome. J. Mol. Evol. **28**: 32–42.

Stevens, T.A., Duffield, D.A., Asper, E.D., Hewlett, K.G., Bolz, A., Gage, L.J., and Bossart, G.D. 1989. Preliminary findings of restriction fragment differences in mitochondrial DNA among killer whales (*Orcinus orca*). Can. J. Zool. **67**: 2592–2595.

Swartz, S.L. 1986. Gray whale migratory, social and breeding behaviour. Rep. Int. Whal. Comm. (Spec. Issue) No. 8. pp. 207–229.

Tomilin, A.G. 1957. Mammals of the USSR and adjacent countries. (Translated from Russian by the Israel Program for Scientific Translations, Jerusalem.)

Wada, S., Kobayashi, T., and Numachi, K.I. 1991. Genetic variability and differentiation of mitochondrial DNA in minke whales. Rep. Int. Whal. Comm. (Spec. Issue) No. 13. pp. 203–215.

Wang, J.Y. 1993. Mitochondrial DNA analysis of the harbour porpoises, *Phocoena phocoena* (L.). M.Sc. thesis, University of Guelph, Guelph, Ont.

Wang, J.Y., and Berggren, P. 1995. Mitochondrial DNA analysis of harbour porpoises (*Phocoena phocoena*) in the Baltic Sea, the Kattegat-Skagerrak Seas and off the west coast of Norway. Paper II. *In* Stocks, status and survival of harbour porpoises in Swedish waters. *By* P. Berggren. Ph.D. thesis, Stockholm University, Stockholm.

Watson, A.P., and Gaskin, D.E. 1985. Observations on the ventilation cycle of the harbour porpoise *Phocoena phocoena* (L.). Can. J. Zool. **61**: 126–132.

Westgate, A.J., Read, A.J., Berggren, P., Koopman, H.N., and Gaskin, D.E. 1995. Diving behaviour of harbour porpoise, *Phocoena phocoena*. Can. J. Fish. Aquat. Sci. **52**: 1064–1073.

Westneat, D.F., Noon, W.A., Reeve, H.K., and Aquadro, C.F. 1988. Improved hybridization conditions for DNA 'fingerprints' probed with M13. Nucleic Acids Res. **16**: 4161.

Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D., and Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. **26**: 375–400.

Yurick, D.B. 1977. Populations, subpopulations and zoogeography of the harbour porpoise, *Phocoena phocoena* (L.). M.Sc. thesis, University of Guelph, Guelph, Ont.

Yurick, D.B., and Gaskin, D.E. 1987. Morphometric and meristic comparisons of skulls of harbour porpoise *Phocoena phocoena* (L.) from the North Atlantic and North Pacific. Ophelia, **27**: 53–75.

© 1996 NRC Canada

Appendix

Fragment size estimates (base pairs) for all endonucleases that produced polymorphic restriction patterns in harbour porpoise mitochondrial DNA.

*Alu*I							*Bgl*II			*Eco*RI				
A	B	C	D	E	F	G	A	B	C	A	B	C	D	E
—	—	—	—	—	—	1 320	No site	17 870	—	—	—	—	—	4 890
990	—	990	990	—	990	990	—	—	9 120	4 720	4 720	4 720	4 720	—
—	—	—	—	870	—	—	—	—	8 470	—	4 720	—	—	—
830	830	830	830	830	830	830				—	—	4 520	—	—
—	710	—	—	—	—	—				4 240	4 240	4 240	—	4 240
620	620	620	620	620	620	620				3 950	—	—	3 950	3 950
560	560	560	560	560	560	560				—	—	—	3 340	—
—	—	—	530	—	—	—				2 640	2 640	2 640	2 640	2 640
450	450	450	—	450	—	—				—	—	—	880	—
—	—	—	430	—	430	430				560	—	—	560	560
—	410	410	—	—	—	—								
400	—	—	400	400	400	400								
360	360	360	360	360	360	360								
—	—	—	—	—	350	—								
4 210	3 940	4 220	4 720	4 090	4 540	5 510	na	17 870	17 590	16 110	16 320	16 120	16 090	16 280

*Hae*III									*Hin*dIII	
A	B	C	D	E	F	G	H	I	A	B
1 990	—	1 990	—	1 990	1 990	1 990	1 990	1 990	8 570	—
—	1 930	—	—	—	—	—	—	—	—	6 670
—	—	—	—	—	1 260	—	—	—	3 680	3 680
—	—	—	1 230	—	—	—	—	—	3 130	3 130
1 160	1 160	1 160	1 160	1 160	—	1 160	1 160	1160	—	2 360
—	—	—	—	—	—	—	—	1 100	520	520
1 000	1 000	1 000	—	1 000	1 000	1 000	1 000	1 000		
920	920	920	920	920	920	—	920	920		
—	—	—	—	870	—	—	—	—		
—	—	—	—	—	—	860	—	—		
810	810	810	810	810	810	810	810	810		
760	760	760	760	760	760	760	760	760		
760	760	760	—	760	760	760	—	760		
720	720	720	720	720	720	720	720	720		
720	720	720	720	720	720	720	720	—		
620	620	620	—	620	620	620	—	620		
—	—	—	610	—	—	—	610	—		
420	420	420	—	420	420	420	420	420		
410	410	410	410	410	410	410	410	—		
—	—	—	340	—	—	—	—	—		
—	—	330	—	—	—	—	—	330		
—	—	—	320	—	—	—	—	—		
290	290	290	290	290	290	290	290	290		
290	290	290	—	290	290	290	290	290		
10 870	10 810	11 200	8 290	11 740	10 970	10 810	10 100	11 170	15 900	16 360

© 1996 NRC Canada

*Hin*fI

A	B	C	D	E	F	G	H	I	J	K	L	M
—	—	—	—	—	2 900	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	1 750
—	—	—	1 430	—	—	—	—	—	—	—	—	—
1 410	1 410	1 410	—	—	—	1 410	1 410	1 410	1 410	—	1 410	1 410
1 340	1 340	1 340	—	1 340	—	1 340	1 340	1 340	1 340	1 340	1 340	1 340
1 270	1 270	1 270	1 270	1 270	1 270	1 270	1 270	1 270	1 270	1 270	1 270	1 270
1 060	1 060	—	1 060	1 060	—	1 060	1 060	—	1 060	1 060	1 060	1 060
—	—	—	—	—	—	—	—	1 050	—	—	—	—
920	920	920	920	920	920	920	920	920	920	920	920	—
—	850	—	—	—	—	—	—	—	—	—	—	—
790	790	790	790	790	790	790	790	790	790	790	790	790
—	—	—	790	—	—	—	—	—	—	—	—	—
740	740	—	—	740	—	740	740	—	740	740	740	740
650	650	650	650	650	650	650	650	650	650	650	650	650
—	—	600	—	—	600	—	—	—	—	—	—	—
—	—	—	—	—	—	—	550	—	—	—	—	—
—	—	—	520	—	—	520	—	—	—	—	—	—
480	—	480	480	480	480	480	480	480	480	480	480	480
—	—	460	—	—	460	—	—	—	—	—	—	—
430	430	—	430	430	—	430	430	—	—	430	430	430
—	420	—	420	420	—	420	420	—	—	—	420	420
390	390	390	390	390	390	390	390	390	390	390	390	390
9 470	10 260	8 300	9 140	8 480	8 450	10 410	10 440	8 290	9 040	8 060	9 890	10 730

*Mbo*I

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
2 460	—	2 460	2 460	2 460	2 460	—	2 460	—	—	2 460	2 460	2 460	2 460	2 460	2 460
—	2 180	—	—	—	—	2 180	—	2 180	2 180	—	—	—	—	—	—
1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700	1 700
—	—	—	—	—	—	—	—	—	—	—	1 380	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	1 290	—	—
—	—	—	1 250	—	—	—	—	—	—	—	—	—	—	—	—
1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160	1 160
1 110	1 110	—	1 110	1 110	1 110	1 110	1 110	1 110	1 110	1 110	1 110	1 110	—	—	1 100
—	—	1 060	—	—	—	—	1 060	—	—	—	—	1 060	—	—	—
—	—	—	—	—	—	—	—	870	—	—	—	—	—	—	—
—	—	840	—	—	—	—	—	—	—	—	—	—	—	—	—
780	780	—	780	780	780	780	—	780	780	780	780	—	780	780	780
—	—	—	—	640	640	—	640	—	—	—	—	—	—	—	—
620	620	620	620	620	620	620	620	620	620	620	620	620	620	620	620
—	—	—	—	—	—	—	—	—	—	—	—	—	—	620	—
—	—	—	—	—	—	—	—	—	600	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	540	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	490
—	—	—	—	—	—	—	—	—	—	—	—	450	—	—	—
430	430	—	430	430	—	430	430	430	430	—	430	430	430	430	430
420	420	420	420	—	—	420	—	420	420	420	420	—	420	420	—
—	—	420	—	—	—	—	—	—	—	—	—	—	—	—	—
360	360	—	360	360	360	—	360	360	360	360	360	360	360	360	360
—	—	360	—	—	360	—	—	—	—	360	—	—	—	—	—
330	330	—	330	330	330	330	—	330	—	330	330	—	330	330	330
280	280	280	280	280	280	280	280	280	280	280	280	280	280	280	280
9 650	9 370	9 320	10 900	9 870	9 800	9 010	9 820	10 240	9 640	9 580	11 030	9 630	9 830	9 700	9 720

© 1996 NRC Canada

*Taq*I												
A	B	C	D	E	F	G	H	I	J	K	L	M
2 230	2 230	2 230	2 230	2 230	2 230	2 230	2 230	2 230	2 230	2 230	2 230	2 230
2 060	2 060	2 060	2 060	2 060	2 060	2 060	2 060	2 060	2 060	2 060	—	2 060
1 840	1 840	—	1 840	1 840	—	1 840	—	1 840	1 840	1 840	1 840	—
—	—	—	—	—	—	—	1 710	—	—	—	—	—
1 550	1 550	1 550	1 550	1 550	1 550	1 550	—	1 550	1 550	1 550	1 550	1 550
—	—	—	—	—	—	—	—	—	—	—	—	1 350
—	—	—	—	—	—	—	—	—	—	—	1 290	—
—	—	—	—	—	—	1 140	—	—	—	—	—	—
—	—	1 070	—	—	1 070	—	1 070	—	—	—	—	—
—	—	—	—	—	—	—	—	—	960	—	—	—
870	870	870	870	870	870	—	870	870	870	—	870	870
—	—	—	800	—	800	—	—	—	—	—	—	—
740	740	740	740	740	740	740	740	740	740	740	740	740
—	—	—	—	—	—	—	—	—	—	—	740	—
710	710	710	—	710	—	710	710	710	710	710	710	710
—	610	—	—	—	—	—	—	—	—	—	610	—
—	—	—	—	—	—	—	—	—	—	560	—	—
530	—	530	530	530	530	530	530	530	530	530	—	530
450	450	450	450	—	450	450	450	450	450	450	450	450
410	410	410	410	410	410	410	410	410	410	410	410	410
—	—	—	—	—	—	—	—	410	—	—	—	—
—	—	—	—	—	—	—	—	—	—	330	—	—
290	290	290	290	290	290	290	290	—	290	290	290	290
11 680	11 760	10 910	11 770	11 230	11 000	11 950	11 070	11 800	12 640	11 700	11 730	11 190

© 1996 NRC Canada

Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake (Lake Pääjärvi)

Lauri Arvola, Paula Kankaala, Tiina Tulonen, and Anne Ojala

Abstract: During the ice-free period, three experiments each of 16–17 days were performed in four enclosures to evaluate the effects of moderate pulses of allochthonous humic matter (5–29% of the ambient dissolved organic carbon concentration) and phosphorus (PO_4-P was increased from 1–2 to 15–20 $\mu g \cdot L^{-1}$) on the plankton. The addition of phosphorus, alone or with humic matter, clearly raised the primary production in summer (54–56%), and to a lesser extent in spring (17–23%) and in autumn (17–58%). A significant correlation existed between primary production and bacterial production, indicating that bacterial production was controlled primarily by organic substrates released by algae rather than by phosphorus or humic matter. In the control enclosure, bacterial production constituted 23–32% of primary production, while with extra phosphorus and humic matter the proportion rose to 59%, indicating that humic matter was used by bacteria as an additional energy and carbon source besides algal exudates. Simultaneous enrichment with phosphorus and humic matter resulted in substantially higher bacterial production than with humic matter alone. In autumn, the addition of humic matter alone increased bacterial production, indicating that humic matter contained more easily utilizable organic material at that point than it did earlier. Some algal taxa responded positively to phosphorus and (or) humic matter enrichment, but the effects on proto- and meta-zooplankton communities were less obvious.

Résumé : Pendant la saison libre de glaces, trois expériences durant chacune 16 ou 17 jours ont été réalisées dans quatre enclos en vue d'évaluer les effets sur le plancton de poussées modérées de matière humique allochtone (5–29% de la concentration ambiante de carbone organique dissous) et de phosphore (PO_4-P, passé de 1–2 à 15–20 $\mu g \cdot L^{-1}$). L'ajout de phosphore, seul ou accompagné de matière humique, a nettement fait augmenter la production primaire en été (54–56%) et, dans une moindre mesure, au printemps (17–23%) et à l'automne (17–58%). Une corrélation significative était manifeste entre la production primaire et la production bactérienne, ce qui indiquait que la production bactériene était contrôlée avant tout par les substrats organiques libérés par les algues plutôt que par phosphore ou la matière humique. Dans l'enclos témoin, la production bactérienne constituait 23–32% de la production primaire, tandis qu'avec l'ajout de phosphore et de matière humique la proportion a monté à 59%, ce qui indique que la matière humique était utilisée par les bactéries comme source additionnelle d'énergie et de carbone en plus des exudats des algues. L'enrichissement simultané avec phosphore et matière humique a donné une production bactérienne nettement plus haute qu'avec la matière humique seulement. À l'automne, l'ajout de matière humique seule faisait monter la production bactérienne, ce qui indique que la matière humique contenait alors des matières organiques plus facilement utilisables qu'auparavant. Certains taxons algaux ont réagi positivement à l'enrichissement avec phosphore et (ou) matière humique, mais les effets sur les communautés proto-et méta-zooplanctoniques étaient moins évidents.
[Traduit par la Rédaction]

Introduction

Owing to the large pool of allochthonous organic matter, humic lakes are physically and chemically different from clear-water lakes, and these properties affect the metabolism, dynamics, and structure of the biota (Wetzel 1983). Because of rapid light attenuation in dark-coloured waters, primary production is confined to shallow surface layers (e.g., Eloranta 1978; Jones and Arvola 1984). The bioavailability of phosphorus is restricted in humic lakes by allochthonous dissolved organic matter (DOM) (e.g., Kämäri et al. 1990; Meili 1992) and its association with iron (Jones et al. 1988). Some observations even suggest that the DOM–iron–orthophosphate interaction may decrease primary productivity and phytoplankton biomass in humic lakes (Jackson and Hecky 1980).

Easily assimilated and metabolized oligomeric and monomeric substrates such as dissolved free amino acids, carbohydrates, and lipids constitute only a small percentage of DOM in humic lakes (Münster and Chrost 1990; Münster et al. 1992). However, although recalcitrant polymeric substances make up the bulk of the aquatic humus (Geller 1983; Münster and Chrost 1990), a high load of humic matter (HM) may favour bacterial growth (Tranvik 1988; Moran and Hodson

Received May 4, 1995. Accepted February 15, 1996.
J12904

L. Arvola,[1] P. Kankaala, T. Tulonen, and A. Ojala.
University of Helsinki, Lammi Biological Station, FIN–16900 Lammi, Finland.

[1] Author to whom all correspondence should be addressed.
e-mail: lauri.arvola@helsinki.fi

© 1996 NRC Canada

Table 1. Physical and chemical data (mean ± SD) for lake Pääjärvi during the experiments.

	Spring	Summer	Autumn
Temperature (°C)	9.6±2.5	20.2±2.0	10.2±0.5
pH	6.8±0.2	7.0±0.2	6.6±0.1
Alkalinity (mmol·L^{-1})	0.25±0.04	0.25±0.00	0.24±0.00
Colour (mg Pt·L^{-1})	93±2	84±2	80±1
DIC (mg C·L^{-1})	2.9±0.2	3.0±0.2	3.0±0.1
DOC (mg C·L^{-1})	10.0±0.6	10.2±0.5	9.6±0.2
POC (mg C·L^{-1})	0.23±0.05	0.23±0.06	0.16±0.02
Total P (μg·L^{-1})	8.6±1.0	14.7±2.4	12.2±0.8
PO_4-P (μg·L^{-1})	1.2±0.4	1.8±1.2	1.7±0.5
Total N (μg·L^{-1})	1278±35	1256±31	1202±84
NO_3-N (μg·L^{-1})	899±38	757±27	803±26
NH_4-N (μg·L^{-1})	7.5±2.6	7.8±3.0	7.5±2.6
Ca (mg·L^{-1})	8.0±0.0	7.9±0.3	8.0±0.0
Mg (mg·L^{-1})	2.4±0.0	2.5±0.0	2.5±0.0
Fe (mg·L^{-1})	0.1±0.0	0.1±0.0	0.1±0.0
Na (mg·L^{-1})	3.2±0.1	3.3±0.0	3.3±0.0
K (mg·L^{-1})	2.1±0.0	2.1±0.0	2.1±0.0

Note: The number of observations for each variable was six, except it was three for cations. The values represent the uppermost 3-m water layer.

1990; Tulonen et al. 1992). The utilization of allochthonous DOM by microheterotrophs as a carbon and energy source is strongly affected by the availability of inorganic nutrients (Benner et al. 1988; Tulonen et al. 1992; Kroer 1993). Schindler et al. (1992) proposed that a high content of DOM may decrease the sedimentation rate of iron, and perhaps also of phosphorus, by suppressing flocculation reactions, in which case the effects of DOM on the availability of nutrients can be even more complicated.

Leaching of DOM and nutrients into rivers and lakes is strongly correlated with rates of water discharge (e.g., McDowell and Likens 1988; Heikkinen 1989; Arvola et al. 1990*b*). Thus, lakes in the boreal zone often receive large inputs of DOM, nitrogen, and phosphorus within short periods in spring, and during heavy spells of rain in summer and in autumn. As terrestrial vegetation and soil–water systems, particularly peat areas, are the primary sources of allochthonous DOM, human activity in the catchment, such as forestry, peat draining, and agriculture, greatly influences the DOM input to lakes. Also, climatic changes or changes in hydrological regime can strongly affect the load of nutrients and DOM to rivers and lakes. According to some authors (e.g., Forsberg 1992) the increase in colour values of rivers and lakes recorded in northern Europe in recent decades is related to increased precipitation (cf. Bergström and Carlsson 1994), particularly to precipitation and runoff in autumn (Ivarsson and Jansson 1994*b*). Simultaneously, concentrations of phosphorus and nitrogen have increased substantially in lakes surrounded by intensively cultivated land areas (e.g., Kauppi 1984). If annual temperatures and precipitation increase in Scandinavia as predicted (Boer et al. 1990; Intergovernmental Panel on Climate Change 1990), the factors controlling the humus and nutrient load from the catchment areas may change, thus leading to an increase in leached DOM and nutrients. This would be the opposite of observations in some areas of southern Canada, where dissolved organic carbon (DOC) concentrations in lakes have decreased during the last two decades owing to lowered precipitation (Schindler et al. 1990).

Despite the increasing attention paid by scientists to humic lakes (see Salonen et al. 1992*c*), knowledge of ecosystem functioning in these lakes is still limited. This is particularly true for large humic lakes; the ecosystems of small lakes have been better studied (e.g., Hessen et al. 1990; Salonen et al. 1992*a*, 1992*b*). In this paper we focus on the metabolic responses as well as on the changes in the pelagic community structure in a boreal humic lake of moderate size after sudden inputs of allochthonous DOM and phosphorus. We aimed to test the following hypotheses: (*i*) that phosphorus enrichment of humic water affects the growth of bacteria more than that of algae, (*ii*) that the extra phosphorus enhances bacterial utilization of humic matter, and (*iii*) that enrichment with phosphorus and humic matter is also reflected further in the food chain.

The data were collected from in situ enclosure experiments where seasonality was considered by carrying out the experiments three times during the ice-free period. Carbon budgets for the euphotic zone, based on these experiments, are presented in Kankaala et al. (1996). The impact of phosphorus and HM input on the productivity of phytoplankton and the microbial food chain was also studied in winter at the low temperatures and low illumination prevailing under the ice cover. These results are presented in Tulonen et al. (1994).

Study site

The study was carried out in Lake Pääjärvi, an oligomesotrophic lake in southern Finland (61°04′N, 25°08′E). The lake has an area of 13.4 km^2 and mean and maximal depths of 14.4 and 87 m, respectively. Its drainage area is 244 km^2 and the mean residence time of water is 3.3 years. Dimixis prevails in the lake and in summer the thermocline lies at around 10 m. During calm and sunny weather, steep thermal stratification may occur near the surface of the lake, usually persisting from a few days to 2–3 weeks. The whole water column is well oxygenated throughout the year (Salonen 1981*a*). The water is coloured by humic substances (Table 1), and since the 1970s the colour values have increased by 30% (Arvola 1991). The concentrations of nitrate, calcium, chloride, potassium, and sodium have also increased. The concentration of phosphorus is very low compared with that of nitrogen (N:P ratio approximately 100), although recently a slight increase in phosphorus concentration has been recorded.

Methods

During the ice-free period in 1991, three field experiments, each lasting for 16–17 days, were performed in the western bay (Pappilanlahti) of the lake, where the water depth is ca. 6–7 m. The first experiment was carried out in May–June (29 May to 12 June), ca. 3 weeks after the breakup of the ice and coinciding with the appearance of thermal stratification of the water column. The second experiment was carried out in July–August (30 July to 14 August) during the warmest time of the year when thermal stratification in the lake was strongest. The last experiment took place in September–October (24 September to 9 October) when the whole epilimnion of the lake was homothermal and the thermocline was eroding.

In each experiment, four polyethylene enclosures (diameter 1.9 m, depth 3.0 m, and volume 8.5 m^3) were placed in the lake. The bottom

© 1996 NRC Canada

of each enclosure was closed with a tightened rope. One enclosure (H) was enriched with humic water (ca. 5% of the volume) collected from the outflow of a bog situated in the drainage area of Lake Pääjärvi. In the second enclosure (P) the original concentration of soluble reactive phosphorus (SRP) (1–2 $\mu g \cdot L^{-1}$) was raised to 15–20 $\mu g \cdot L^{-1}$ with KH_2PO_4. The third enclosure (HP) was enriched with humic water as well as with phosphate in the same concentrations as mentioned above. The fourth enclosure (C) without any additions served as a control, and the lake (L) served as a reference for the control. A wooden frame was built around the enclosures to protect them from waves, and the construction was covered with a coarse net hanging at a height of 2 m to keep waterfowl away. The enclosures were installed and the additions were made a day before sampling was started. The increments of nutrients and total organic carbon (TOC) with the added humic water are given in Table 2, and the atomic ratios of carbon, nitrogen, and phosphorus in the lake as well as in the added humic water are given in Table 3.

Samples were taken every 3rd day at 10:00–12:00. Water temperature and dissolved oxygen concentration were measured with a temperature-compensated dissolved oxygen probe, model YSI 57. Total irradiance ($J \cdot m^{-2}$) was measured continuously throughout the experiments with a Kipp and Zonen solarimeter ($W \cdot m^{-2}$). The penetration of photosynthetically active radiation (PFFR, $\mu mol \cdot m^{-2} \cdot s^{-1}$) was measured 2–3 times per experiment with a Li-Cor Li 1000 light meter equipped with a spherical quantum sensor. Samples for most chemical and biological analyses were taken with a 0.3 m long (2 L) acrylic and Teflon (stoppers), or coated (metal parts), tube sampler (Limnos) so that three consecutive samples were combined, i.e., the sample depths were ca. 0–1, 1–2, and 2–3 m. Dissolved nutrients were determined immediately after sampling by filtering the samples through 0.2-µm Millipore filters, rinsed with deionized water and sample water. Inorganic phosphorus (Murphy and Riley 1963), nitrate–nitrite (Wood et al. 1967), and ammonium (Solorzano 1969) were determined with an Akea autoanalyzer. Subsamples of unfiltered water were frozen for later determinations of total phosphorus and nitrogen with persulphate digestion (Koroleff 1979). Water pH, alkalinity (Gran titration), electrical conductivity, and colour (platinum was used as a reference) were measured every 3rd day. Samples for cations (Ca, Mg, Fe, Na, K) were taken at the beginning and at the end of each experiment and determined with AAS. Chloride and sulphate were determined by ion chromatography (Dionex) and particulate organic carbon (POC) and DOC by a high-temperature combustion method (Salonen 1979). POC was analysed from material collected on preignited Whatman GF/F filters and DOC from the filtrates stored frozen (–20°C) in precombusted (450°C) glass vials.

Phosphatase activity was measured with a Hitachi F-4000 fluorescence spectrophotometer using 100 $\mu mol \cdot L^{-1}$ 4-methylumbelliferyl as a substrate and 4-methylumbelliferone for calibrations. All measurements were carried out within 0.5–3 h of sampling, and before measurement the darkened samples were kept at the ambient temperature (±2°C) in acid-washed and precombusted scintillation vials. For the assay a 2.9- mL subsample was incubated with the substrate (33 nM) for 10–30 min (time-scanning 330 nm for excitation and 445 nm for emission according to Münster et al. 1989). For the measurements, samples were pooled (0–3 m), except when vertical differences in phosphatase activity were checked. Samples for measurements were not filtered except in spring when the assay was also done using filtered samples (1.0-µm Nuclepore filters).

Primary production of phytoplankton was measured with the radiocarbon technique (Schindler et al. 1972). Samples were incubated in preignited 130-mL Duran-glass bottles with $Na_2{}^{14}CO_3$ solution (specific activity 285 $kBq \cdot L^{-1}$) for 24 h. The isotope solution was made of $Ba^{14}CO_3$ according to the procedure suggested by Steeman Nielsen (1952; see also Salonen and Arvola 1986). The incubation depths were as follows: 0.5 m for the sample from 0–1 m, 1.5 m for 1–2 m, and 2.5 m for 2–3 m. At the end of the incubation period, triplicate subsamples of 6 mL were transferred to 20-mL scintillation vials with a drop of 10% phosphoric acid. The inorganic ^{14}C was evaporated in a vacuum hood for 48 h, after which 9 mL of scintillation liquid (OptiPhase3) was added and the radioactivity was measured with a Wallac Ultrobeta 1210 scintillation counter. Quenching was corrected with external standardization. Exudation by phytoplankton was measured as incorporated ^{14}C in filtrates, passed through 1-µm Nuclepore filters. The measurement was carried out only from the uppermost 0- to 1-m samples and the proportion of production extracellularly released was then assumed to be the same also at 2- to 3-m depths. The concentration of dissolved inorganic carbon (DIC) was analysed with an infrared carbon analyzer according to Salonen (1981*b*).

Chlorophyll *a* concentration was determined with a Hitachi F-4000 fluorescence spectrophotometer using excitation and emission wavelengths of 430 and 671 nm. Water samples of 500–1000 mL were filtered onto Whatman GF/C filters and stored frozen (–20°C) before analysis. Pigments were extracted in 7 mL of 96% ethanol, and to increase the efficiency of extraction and to denature chlorophyllase enzymes (Jeffrey and Hallegraeff 1987) samples were homogenized for 3 min with an ultrasonic cell disruptor.

Samples for phytoplankton and protozoa were preserved with glutaraldehyde to a final concentration of 0.3% and stored in a dark, cold (4°C) room. The species composition and biomass of algae and protozoa were determined with a Nikon Diaphot and a Wild M 40 inverted microscope using Utermöhl's (1958) technique. Samples of 50 mL were sedimented for 48 h and 25–50 random fields from each sample were counted using a 40× objective (all cells) and another 25–50 fields were counted with a 20× objective (large cells). Biovolumes were estimated from linear dimensions measured for each species. The conversion factor from biovolumes to carbon was 0.2 pg $C \cdot \mu m^{-3}$ (cf. Rocha and Duncan 1985; Putt and Stoecker 1989; Verity et al. 1992).

Bacterial production was measured with the [^{14}C]leucine incorporation method as described by Tulonen (1993). Triplicate samples of 5 mL in 20-mL glass vials were incubated for 60 min with 30 nM of [L-^{14}C]leucine (12.7 $kBq \cdot nmol^{-1}$). Samples treated with glutaraldehyde to a final concentration of 4% served as blanks.

For the determination of density and biomass of bacteria, samples of 5–10 mL were preserved with glutaraldehyde to a final concentration of 2%. They were filtered onto black 0.2-µm Nuclepore filters and stained with particle-free acriflavine solution (10 nM; Bergström et al. 1988). Cells were counted and sized under a Nikon Optiphot epifluorescence microscope (100× oil immersion objective, exitation filter 450–480 nm). From each sample, counting continued until at least 20 fields of view had been counted or until the standard error of counted fields was <10%. Biomass of bacteria was estimated by measuring the length and width of at least 100 cells relative to the circles of a calibrated eyepiece graticule (Petterson Globe and Circle, GI, Eyepiece Graticules Ltd.). Bacterial cell volumes were converted to carbon by a factor 0.36 pg $C \cdot \mu m^{-3}$ (Tulonen 1993).

Metazooplankton was sampled with the 0.3-m tube sampler (2 L) from the surface to 3 m depth and the nine samples obtained were pooled and concentrated with a 50-µm mesh net. Samples were preserved in cold sucrose and formaldehyde (final concentration 4%) according to Prepas (1978). Species were identified and individual numbers were counted with an inverted microscope at 60× magnification. Samples too dense for direct counting were divided with a Folsom splitter and one half or one fourth of the sample was counted.

Biomass of zooplankton was analysed from an integrated sample (0–3 m) taken from the lake at the beginning, and from the lake and each enclosure at the end of the experiment. Samples were immediately preserved in 2% formaldehyde and frozen at –20°C (Salonen and Sarvala 1985). The individual carbon content of dominant crustaceans and their developmental stages (n = 24–425/taxon) were determined with a high-temperature combustion method (Salonen 1979). As the differences in the individual carbon contents between the treatments were not statistically significant, the crustacean

© 1996 NRC Canada

biomass was estimated by multiplying the number of individuals of a species or developmental stage by its average carbon content for each experimental period. The rotifer biomass was estimated by using the carbon values of Latja and Salonen (1978) and Salonen and Latja (1988) for rotifers in Lake Pääjärvi.

Community respiration was measured as the increase in DIC after 24-h incubation in 50-mL acid-washed and preignited glass bottles wrapped in aluminium foil. The incubation depths were the same as in primary production measurements. DIC was analysed with an infrared carbon analyzer according to Salonen (1981*b*).

Sedimentation rates were measured with sets of three acrylic tubes 0.6 m in length and 0.1 m in diameter. They were suspended vertically at the bottom of each enclosure and at the corresponding depth in the lake and were left there throughout the experiment (16–17 days). Subsamples for analyses of dry weight and carbon, nitrogen, and phosphorus were frozen (–20°C) for later measurements carried out as described above.

For statistical analyses the data were log-transformed to meet normality assumptions (parametric analyses), but if this presumption was not fulfilled nonparametric analyses were applied. The statistical packages of SYSTAT 5.0 and Excel 5.0 were used to analyse the data.

Results

Physical and chemical properties

The differences in mean temperatures between the treatments were usually <0.5°C. In spring the surface water temperature rose in the enclosures and in the lake from 6.8 to 14.5°C (Fig. 1), and the uppermost part of the water columns stratified thermally during the experiment. In summer the temperature fluctuated around 20°C (Fig. 2). The maximum surface temperature (24.1°C) was recorded at the beginning of August when the temperature difference between the surface and the bottom (3 m) of the enclosures was 4.3–5.1°C and the thermal stratification was most pronounced. In autumn the water column was homothermal, and over the course of the experiment temperature dropped only from 11.0 to 9.4°C (Fig. 3).

Irradiance varied widely within the experiments as well as between them (Figs. 1–3). In the humic water of Lake Pääjärvi, light penetration is naturally poor, and by HM addition the light conditions further deteriorated as the colour of water increased by 27–51%. This was especially clear during the summer and autumn experiments, when the photon flux fluence rate (PFFR) at 1 m depth was 38–50% lower in the H and HP enclosures than in the lake. At the beginning of these experiments only 13–16% of the subsurface PFFR penetrated to 1 m. Within a week most of the added HM had sedimented or otherwise disappeared (Figs. 1–3), and the amount of light almost equalled that in the other enclosures and in the lake. The enclosed water columns covered almost the whole euphotic layer as only 1–2% of PFFR remained at the bottom of the enclosures. In the lake 2–3% of the subsurface PFFR reached the corresponding depth.

The concentration of DOC in the lake varied from 9.6 to 10.2 mg $C \cdot L^{-1}$ (Table 1), and the addition of humic water increased it by 5–29% (Table 2). Most of the added organic matter was in dissolved form. However, in summer, particles (POC) represented a higher proportion of the added HM (Table 2), and this caused a 3.3-fold increase in the concentration of POC, although most of the added particles sedimented within the first few days.

The added PO_4 in the P and HP treatments disappeared quickly so that after 9 days (in summer less than 9 days) the concentrations of soluble phosphorus were the same as in other treatments (Figs. 1–3). This indicates a rapid uptake of phosphorus by algae and bacteria and (or) abiotic disappearence through sedimentation and adsorption. Some extra phosphorus also came with the added humic water (Table 2), which increased the concentration of SRP by 0.7–1.4 $\mu g \cdot L^{-1}$ (20–35% of phosphorus in humic water was in the dissolved form), so that the ambient concentration of SRP was doubled and total phosphorus increased by ca. 10%. On the other hand, by dilution the addition of humic water decreased the concentration of NH_4 from 10–12 to 5–7 $\mu g \cdot L^{-1}$. However, the ratio of inorganic nitrogen to phosphorus remained >300, a clear indication of phosphorus rather than nitrogen limitation. Compared with the lake water, the added humic water consisted, particularly in spring and summer, of much more phosphorus in relation to carbon, but much less nitrogen (Table 3).

Phosphatase activity

In spring, phosphatase activity varied from 0.3 to 55 $nmol \cdot L^{-1} \cdot h^{-1}$ (Fig. 4) and showed an increasing trend in all treatments, which coincided with the increase in temperature above ca. 10°C. The differences between the enclosures or between different depths were not significant (ANOVA). Most (80–100%) of the activity was found in the size fraction <1 μm and was probably more related to bacteria than algae. In summer, phosphatase activity was lower than in spring, varying between 0.0 and 5.5 $nmol \cdot L^{-1} \cdot h^{-1}$, and no changes could be detected during the experiment. In autumn no activity could be found, although the measurements were repeated four times, and the incubation time was lengthened to 30 min.

Primary production and chlorophyll *a*

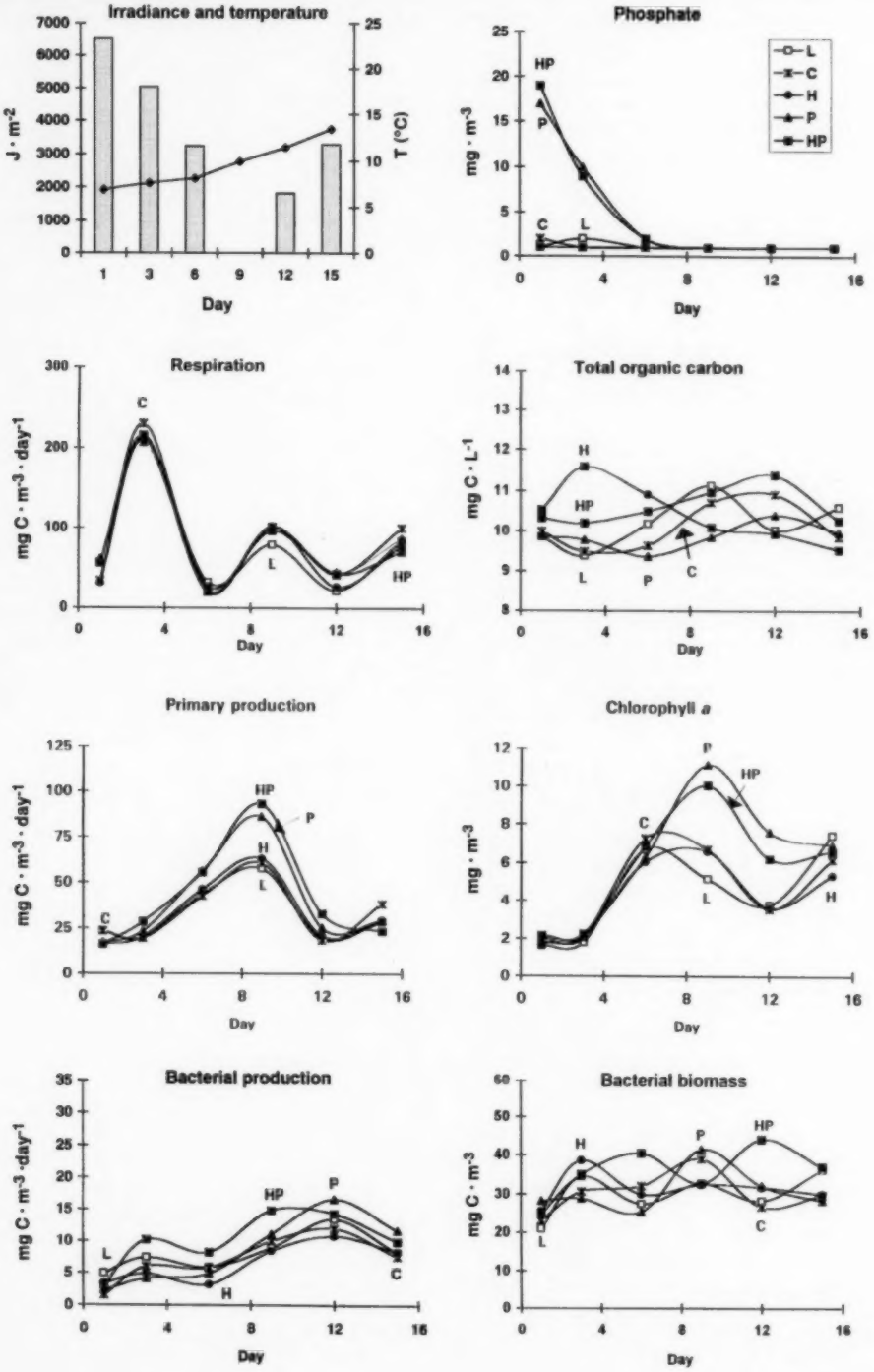
The highest daily primary production was measured in summer and the lowest in autumn (Figs. 1–3). In comparison with the control, primary production was on average 17–60 and 19–57% higher in enclosures with phosphorus or combined phosphorus and HM additions, respectively. In spring a distinct peak in primary production was observed in the middle of the experiment simultaneously in all treatments, and the highest primary production was found in HP (53% more than in control) and in P (41%) enclosures. In summer primary production was highest during the first half of the experiment and, as in spring, the highest values were recorded in P and HP enclosures. In autumn no peaks in primary production were observed, but the values were again highest in P and HP enclosures.

Vertical diminution of primary production was clear in all treatments, but most distinct in the enclosures with added HM. At 1.5 m depth in the lake, primary production was on average 52% of that at 0.5 m. In the enclosures with and without HM additions, the corresponding values were 38–39 and 43–44%, respectively.

There were no significant differences between the treatments in the proportions of primary production extracellularly released, which ranged from 16 to 32%. Some seasonal variation was found; the proportion was highest in summer (on average 31%) and lowest in autumn (19%). Variation in chlorophyll *a* concentration was similar to that in primary production, and the highest values were always found in enclosures P and HP (Figs. 1–3). The ratio of primary production to chlorophyll *a*

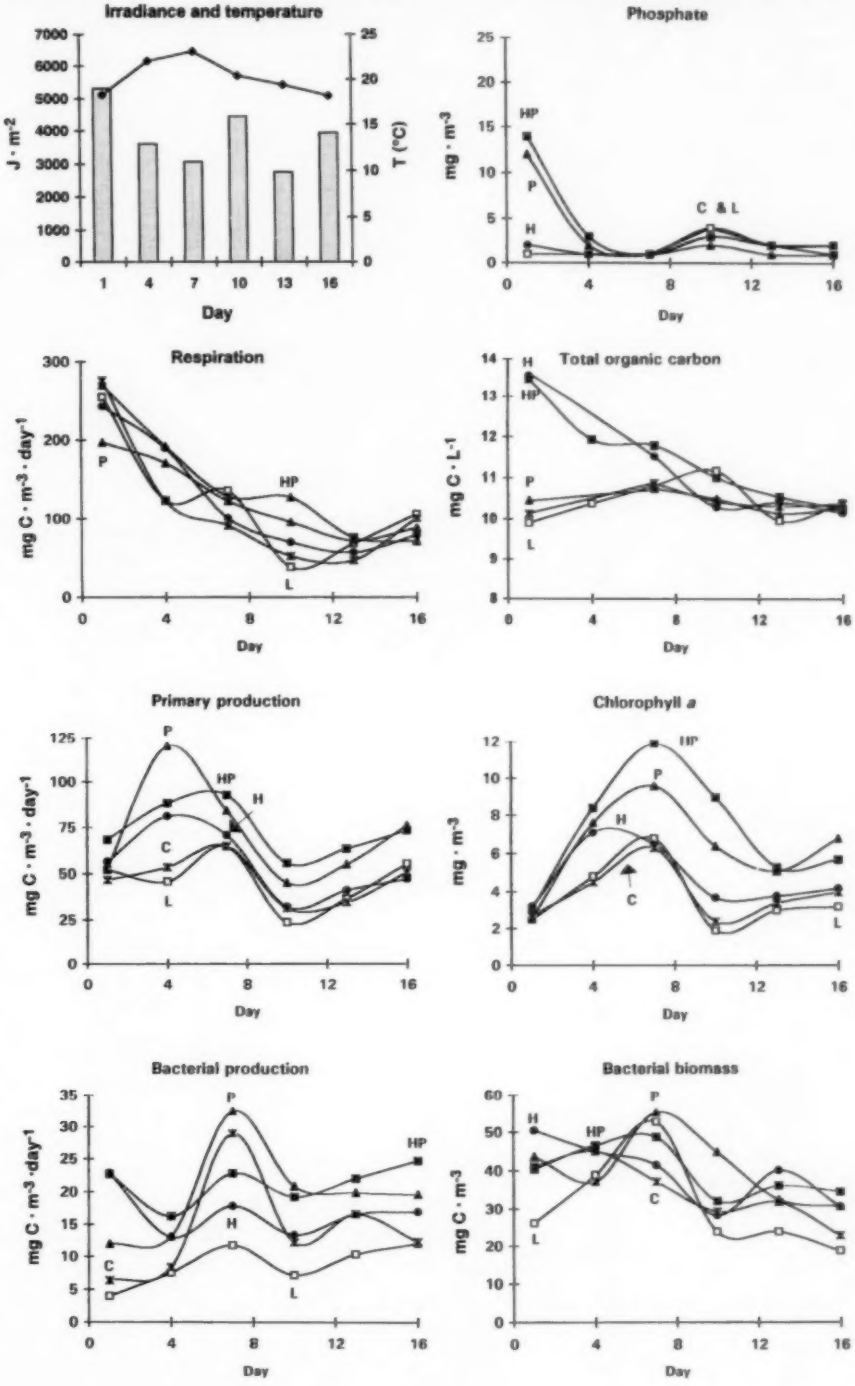
© 1996 NRC Canada

Fig. 1. Changes in physical, chemical, and biological variables during the spring experiment. Irradiance (bars) is given in integrated daily values during the primary production incubations.



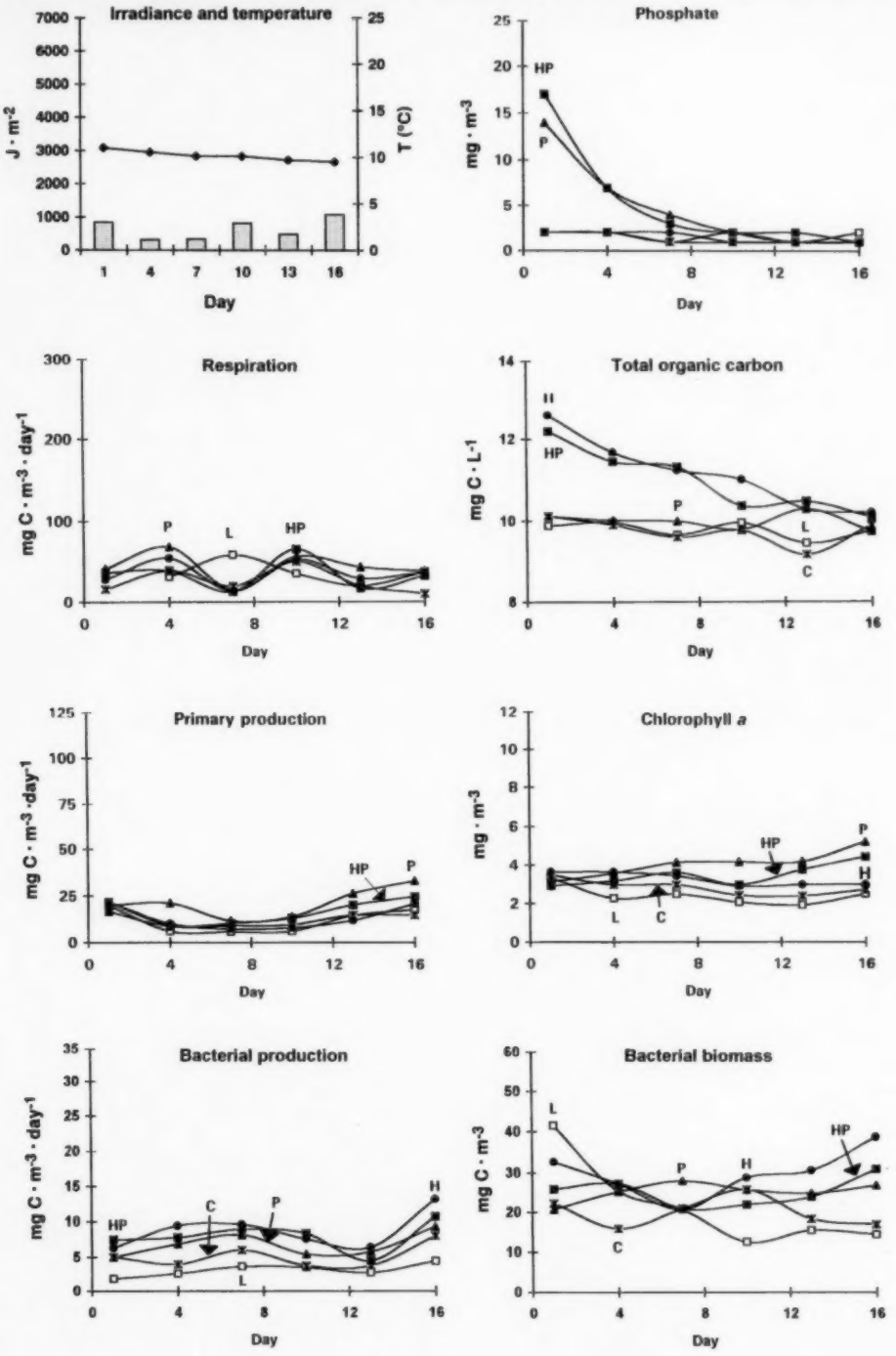
© 1996 NRC Canada

Fig. 2. Changes in physical, chemical, and biological variables during the summer experiment. Irradiance and symbols are as in Fig. 1.



© 1996 NRC Canada

Fig. 3. Changes in physical, chemical, and biological variables during the autumn experiment. Irradiance and symbols are as in Fig. 1.



© 1996 NRC Canada

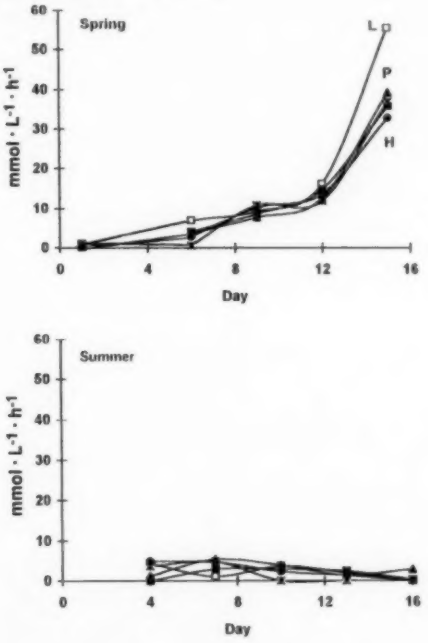
Table 2. The increment of organic carbon, nitrogen, and phosphorus per litre in the enclosures with added humic water, and the proportion (%) of different fractions of the constituents in the added humic water.

	Spring	Summer	Autumn
Organic carbon (mg C·L^{-1})	0.6	3.7	2.7
DOC (%)	90.6	79.3	95.6
POC (%)	9.4	20.7	4.4
Nitrogen (μg·L^{-1})	33	98	56
NO_3+NO_2-N (%)	1.6	0.8	1.0
Phosphorus (μg·L^{-1})	1.0	8.5	3.5
PO_4-P (%)	35	20	33

Table 3. The atomic ratios of carbon, nitrogen, and phosphorus in the lake water as well as the added humic water during different experiments.

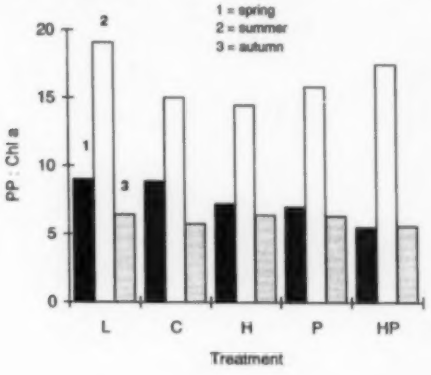
	Spring	Summer	Autumn
Lake water			
C:N	9.1	9.5	9.3
C:P	3004	1793	2033
N:P	329	189	218
Humic water			
C:N	21	44	56
C:P	1550	1125	1993
N:P	73	26	35

Fig. 4. Phosphatase activity in the enclosures and in the lake during the spring and summer experiments.



was highest in summer and lowest in autumn but was not significantly different between the treatments (Fig. 5). Chlorophyll *a* alone explained 47–59% of the variation in primary

Fig. 5. Ratio of mean primary production (PP; mg C·m^{-3}·day^{-1}) to mean chlorophyll *a* concentration (mg·m^{-3}) in the experimental enclosures and in the lake.



production in the enclosures, and when phosphate, ammonium, and irradiance were included in multiple regression analyses, 57–82% of the variation could be explained.

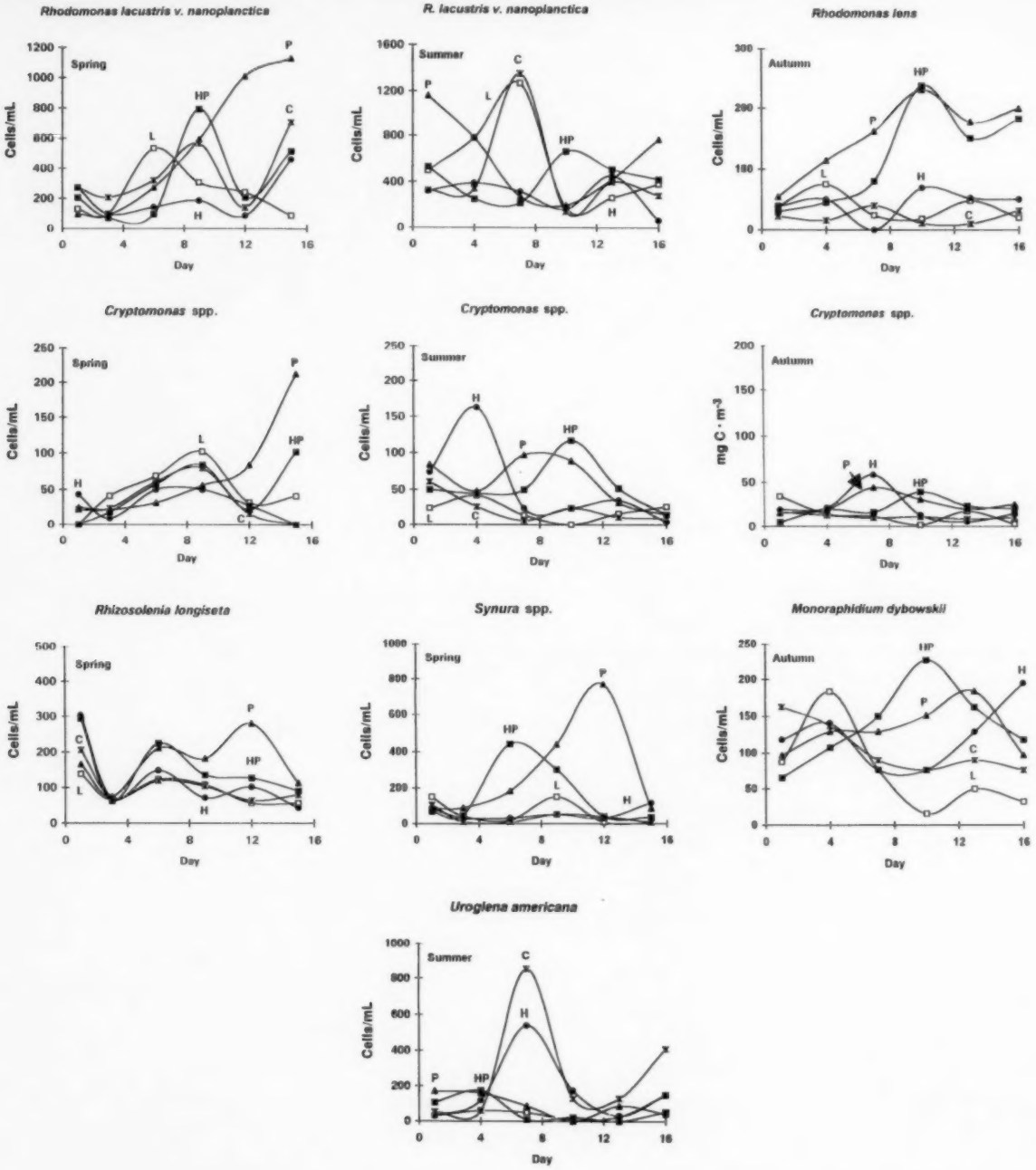
Phytoplankton biomass and species composition

In the spring and summer experiments the biomass of algae varied in parallel with the chlorophyll *a* concentration and primary production, and the highest biomasses (max. 260 mg C·m^{-3} in spring and 240 mg C·m^{-3} in summer) were found in enclosures P and HP. In autumn the algal biomass was lower and showed less variation between the treatments.

Perhaps owing to the relatively short duration of the experiments, only minor changes in the species composition of phytoplankton were observed. In spring, when cryptophytes, chrysophytes, and the diatom *Rhizosolenia longiseta* dominated, the cell number of *Rhodomonas lacustris* v. *nannoplanctica* (Cryptophyceae) increased significantly, especially in enclosure P (Fig. 6). Besides *Rhodomonas lacustris* v. *nannoplanctica, Synura* spp. (Chrysophyceae) showed a pronounced response to phosphorus enrichment. Cryptophytes were also abundant in the summer experiment, in which *Rhodomonas lacustris* v. *nannoplanctica* showed an immediate response to phosphate enrichment; even in the first sampling, ca. 20 h after the onset of the experiment, the cell number was 2–3 times higher in enclosure P than in other treatments. However, after this rapid growth phase the population collapsed. At the same time the cell number of *Rhodomonas lacustris* v. *nannoplanctica* increased quickly in the control enclosure and in the lake. The highest cell numbers of *Cryptomonas* spp. were always recorded in the enclosures treated with phosphorus or HM (Fig. 6). The densities of the colonial chrysophytes *Uroglena* spp. and *Dinobryon* spp. were lowest in enclosure P, whereas two chlorophytes, *Chlamydocapsa planctonica* and *Oocystis parva*, had an opposite response and had their highest cell numbers in enclosure P. In autumn *Rhodomonas lens* responded equally strongly to phosphorus and HM + phosphorus additions. In this last experiment the cell numbers of *Rhodomonas lacustris* v. *nannoplanctica*, *Cryptomonas* spp., and *Asterionella formosa* (Diatomophyceae) did not vary between the treatments. The cell number of *Monoraphidium dybowskii* (Chlorophyceae) slightly increased

© 1996 NRC Canada

Fig. 6. Densities of dominant algal species in the enclosures and in the lake during different experiments.



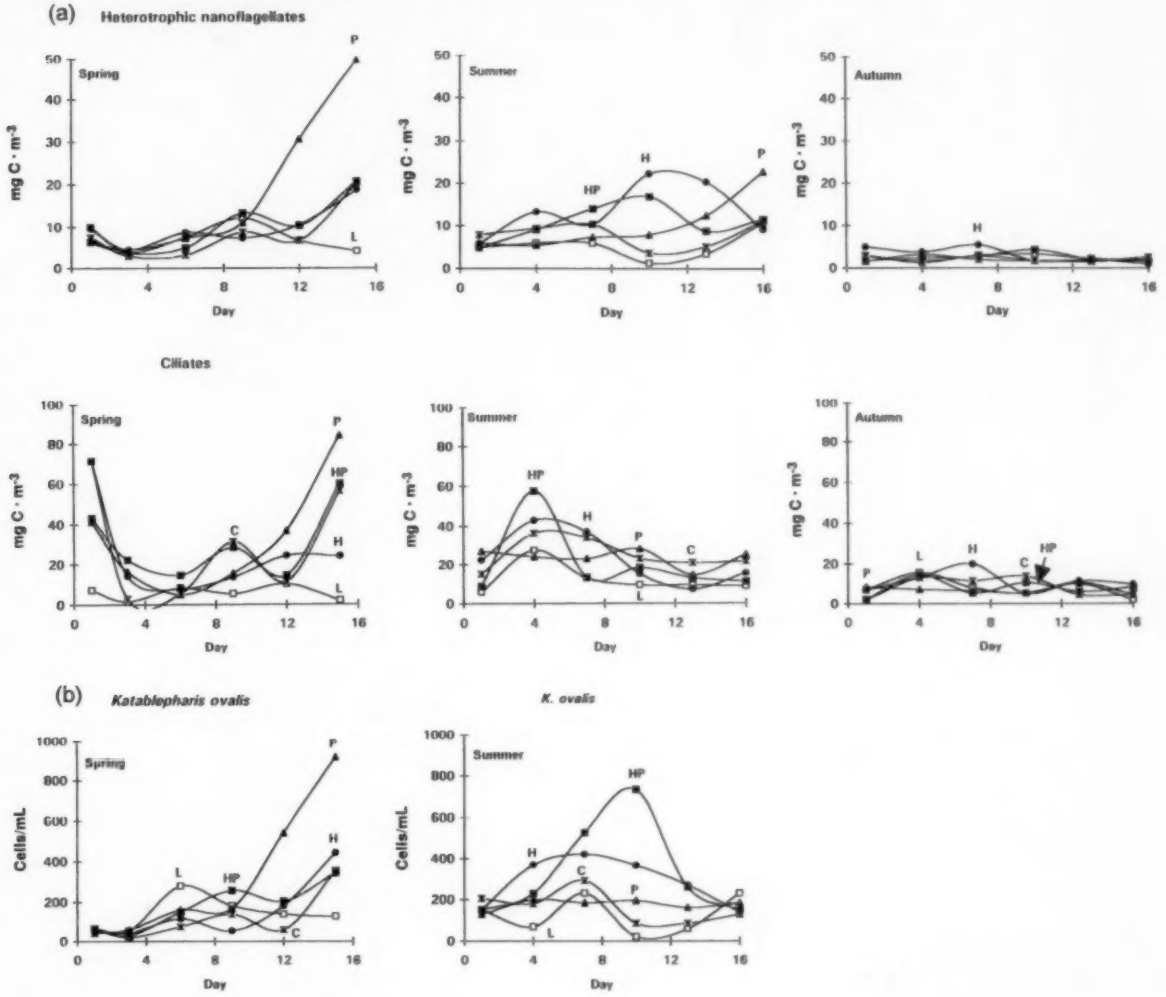
in the enclosures with HM and (or) phosphorus additions (Fig. 6), but decreased in the lake and in the control.

Bacterioplankton

In all experiments bacterial production was highest in the enclosures HP and (or) P, being enhanced by 40–57 and 18–39%, respectively, in comparison with the control (Figs. 1–3). In summer and in autumn bacterial production was also enhanced by HM alone (17–73% higher than in the control). In spring bacterial production showed an increasing trend in all

© 1996 NRC Canada

Fig. 7. Heterotrophic flagellates (HNF) and ciliates in the enclosures and in the lake during the experiments: (*a*) biomass fluctuations, (*b*) density of *Katablepharis ovalis* (Cryptophyceae, HNF).



treatments (Fig. 1), but in summer and in autumn such a pattern was not found (Figs. 2–3). The highest values of bacterial production were measured simultaneously or after the maxima in primary production, but in spring and in summer the addition of HM and phosphorus resulted in a slightly increased bacterial production before clear responses in phytoplankton production were obvious. In summer, bacterial production was on average twice as high as in spring or in autumn.

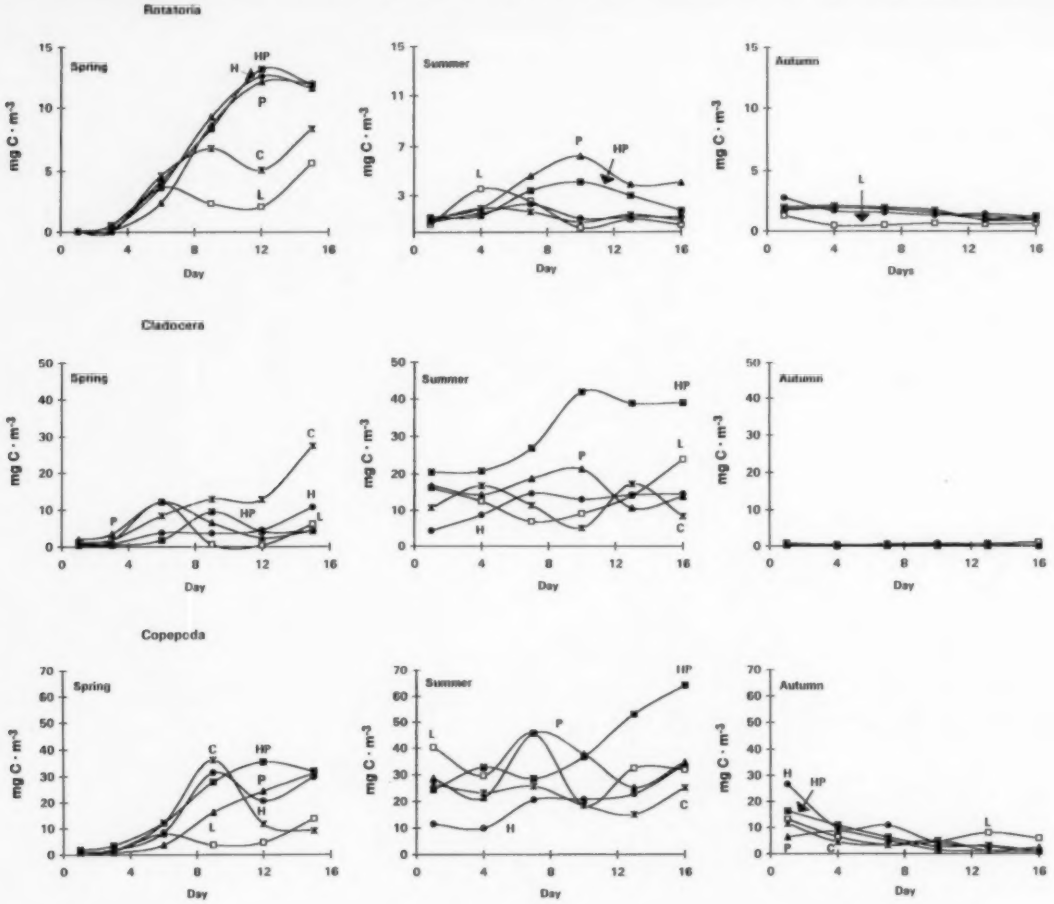
The bacterial cell numbers varied from 2.4 to 5.9 × 10^6 cells/mL in spring and in summer, and from 1.8 to 4.1 × 10^6 cells/mL in autumn. Although bacteria were smallest in summer (mean sizes 0.022 μm^3 in summer, 0.028 μm^3 in spring, and 0.026 μm^3 in autumn), the mean biomass of bacteria was highest in summer and lowest in autumn (Figs. 1–3). No trends in biomass development could be detected except in summer, when the biomass declined in all treatments. The density and biomass of bacteria was in general highest in the enclosures HP and P, and in autumn also in the enclosure treated with HM, but the averages of the treatments did not differ significantly (ANOVA) from each other.

Protozoa

In the spring experiment the majority of protozoan biomass consisted of ciliates (Fig. 7*a*). After the onset of the experiment the numbers decreased in all enclosures and in the lake but later increased, except in enclosure H. During the first half of the spring experiment the biomass of heterotrophic nanoflagellates (HNF) was the same in all treatments. Later the biomass in enclosure P increased from ca. 10 to 50 mg C·m^{-3}, whereas similar changes could not be found in other treatments. In summer, ciliate biomass was highest (especially in enclosure HP) during the first half of the experiment, but HNF biomass increased in the second half of the experiment, especially in the manipulated enclosures. In autumn, ciliate biomass was again

© 1996 NRC Canada

Fig. 8. Biomass fluctuations of metazoa (Rotifera, Cladocera, and Copepoda) in the enclosures and in the lake during different experiments.



slightly higher than that of HNF, and the variation in the course of the experiment was smaller than in spring or in summer. Significant differences between the treatments could not be found in protozoan biomass in general, but the biomass of HNF was higher than in the control in spring in enclosure P.

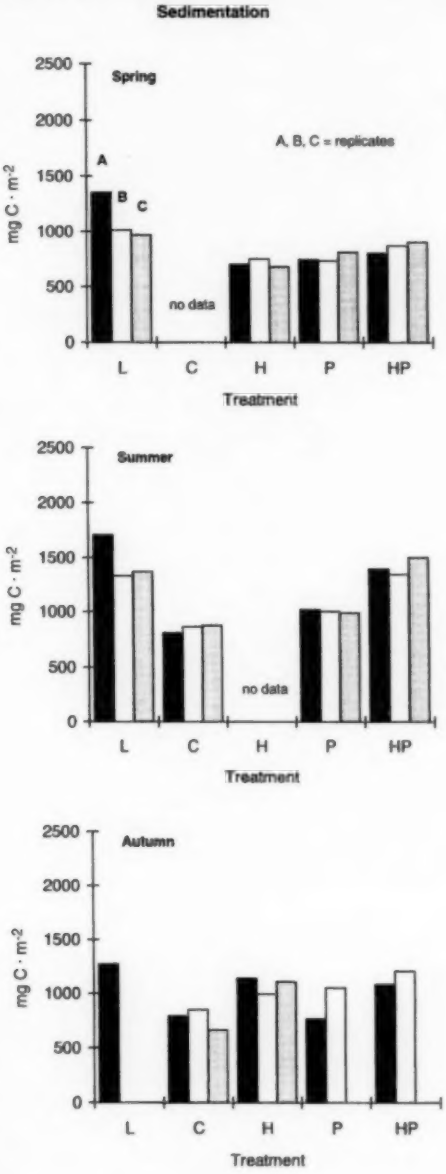
Among HNF, unidentifed cells less than 6 μm in length dominated and cell numbers up to 3300 cells/mL were recorded. Choanoflagellates such as *Desmarella moniliformis*, *Monosiga* spp., *Aulomonas purdyi*, *Stelexmonas dichotoma*, and *Salpingoeca frequentissima* were frequently abundant (up to 250 cells/mL) in all enclosures. One heterotrophic flagellate, *Katablepharis ovalis* (Cryptophyceae), had a high vernal density in enclosure P (up to 900 cells/mL), whereas in summer its highest densities were recorded in the H and HP enclosures (Fig. 7*b*). Members of oligotrich genera, such as *Halteria* sp. and *Strombidium* sp. (size 10–40 μm), were usually the most numerous ciliates. Other taxa such as the flagellates *Gyromitus cordiformis* and *Paramastix conifera* as well as a peritrich ciliate, *Vorticella* sp., an oligotrich ciliate, *Tintinnopsis* sp., and a testate amoeba, *Difflugia* sp., were regularly found as well as several small (<10 μm) and large (length up to 80 μm) unidentified ciliates. At the end of the spring experiment a large (length up to 250 μm) unidentified amoeba was found in all enclosures, with particularly high numbers (up to ca. 100 cells/mL) in those treated with HM. In spring the daily average volumes of ciliates were higher (5666 ± 3205 μm^3) than in summer (2178 ± 1638 μm^3) (*t* test, $P < 0.001$).

Metazooplankton

At the onset of the spring experiment the density and biomass of metazooplankton was very low (Fig. 8) but it rapidly increased towards the end of the experiment (up to 189–411 individuals/L and 47–56 mg C·m^{-3} in the enclosures, and up to 190 individuals/L and 25 mg C·m^{-3} in the lake). Rotifers (*Keratella cochlearis*, *Kellicottia longispina*, *Conochilus* spp., *Polyarthra vulgaris*, and *Asplanchna priodonta*) grew most vigorously in the enclosures treated with HM and (or) phosphorus, and during the 2nd week of the experiment these species comprised 20–35% of the total biomass of metazooplankton. Copepods made up more than half of the biomass in all treatments, except in the control enclosure where a cladoceran (*Bosmina longispina*) increased rapidly during the second half of the experiment. *Eudiaptomus gracilis* and *Thermocyclops oithonoides* were the most abundant copepod

© 1996 NRC Canada

Fig. 9. Sedimentation of carbon in different treatments and in the lake during the experiments.



species. *Limnocalanus macrurus*, a cold stenotherm species, appeared regularly but at low densities.

At the beginning of the summer experiment the biomass of metazooplankton ranged from 40 to 57 mg C·m^{-3}. This was approximately the same as at the end of the spring experiment in all enclosures except H where, probably coincidentally, the biomass was only half that in the other treatments. In the lake and in the control the total biomass did not vary much during the experiment. Rotifers increased steadily until day 10 in enclosures P and HP, where the biomass (mainly *Kellicottia longispina*, *Conochilus* spp., and *Polyarthra* spp.) was higher than in the other treatments (ANOVA, Tukey's honestly significant difference test, $P < 0.05$). At the end of the summer experiment the zooplankton biomass was about twice as high (105 mg C·m^{-3}) in the HP enclosure as in other treatments. This was partly due to a rapid growth of cladocerans, *Daphnia cristata*, *Diaphanosoma brachyurum*, and *B. longispina*, which had higher biomass in the HP enclosure than in other treatments (ANOVA, Tukey's honestly significant difference test, $P < 0.01$); the biomass of copepods (*E. gracilis* and *T. oithonoides*) increased more in this enclosure than in the other treatments.

In autumn the initial biomass of zooplankton varied between 9 and 18 mg C·m^{-3} except in enclosure H, where the starting biomass was higher. At this time reproduction had already ceased and in all treatments the biomass of zooplankton decreased over the course of the experiment. The most abundant species were the rotifers *Keratella cochlearis* and *Kellicottia longispina*, although most of the biomass consisted of the late copepodite stages and adults of *E. gracilis*, as well as stages CIV–V of cyclopoids (most abundant species, *T. oithonoides*). A few male and ephippial females of cladocerans were observed during this experiment.

Community respiration

In spring no distinct differences in respiration rates between treatments were found (Fig. 1). In summer and autumn the respiration values were slightly higher in the manipulated enclosures (H, P, HP) than in the control, but the differences were not significant (*t* test, cf. Figs. 2–3). Both in spring and in summer, respiration decreased during experiments but was correlated with neither daily production nor biomasses of plankton. Because of the relatively high initial concentration of DIC in Lake Pääjärvi (Table 1), the precision of the measurement within 24-h incubations was not as good as in humic lakes with low concentrations of DIC (Salonen et al. 1983). In agreement with earlier observations of boreal humic lakes (e.g., Salonen et al. 1983) the highest respiration values were measured in summer (average 115–144 mg C·m^{-3}·day^{-1}), and the lowest in autumn, corresponding with the changes in productivity of the pelagic community.

Sedimentation

The sedimentation rate was highest in the lake, probably owing to the resuspension of the bottom material in this relatively shallow bay. In enclosure HP the sedimentation rate was almost comparable to that of the lake, whereas in enclosure P the sedimentation rate was lower (Fig. 9). In the lake 10–15% of the sedimented dry mass (DM) consisted of organic carbon, whereas in enclosures this proportion varied between 15 and 26%. The highest percentages were recorded in enclosures H and HP. In the settled material the atomic ratio of C:P was always very high (up to 840), and the lowest ratios were found in the enclosures treated with phosphorus. After phosphorus additions the atomic ratio of N:P was quite close (12:1 to 19:1) to the Redfield ratio (16:1) for phytoplankton, which was in contrast to the N:P ratio in the lake (189–329) and smaller than that in the humic water (26:1 to 73:1) added into the enclosures. The atomic ratio of C:N varied from 14:1 to 22:1 and was clearly higher than the Redfield ratio (6.6).

In summer the sedimentation of phosphorus represented

© 1996 NRC Canada

Fig. 10. Relationship between mean phytoplankton production (PP; ±SD) and initial phosphorus (P) concentration in the enclosures and in the lake during different experiments.

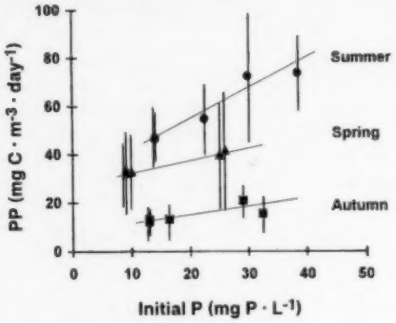
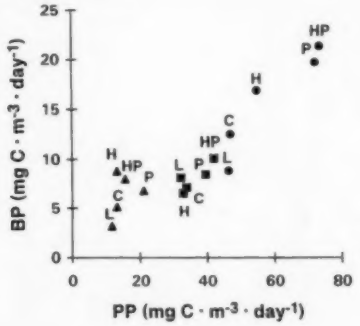


Fig. 11. Relationship between mean primary (PP) and bacterial production (BP) in the euphotic zone in the enclosures and in the lake during the experiments.



only 1.4% of the added phosphate in enclosure P, but in autumn it constituted 7.4%. When both HM and phosphorus were added, the sedimentation of phosphorus represented 6–12% (more in summer and less in autumn) of the added phosphorus (phosphate and phosphorus linked with HM).

Discussion

Enclosures have proven useful for studying the responses of aquatic ecosystems to nutrient enrichments, food web manipulations, and toxic substances (see O'Brien et al. 1992 for a review). However, the limitations of the approach including shading, reduced turbulence, and growth of periphytic algae on the walls of the enclosures were evident to some extent in this study. In general, however, the physical and chemical conditions, and the metabolic processes such as primary production and community respiration in the control enclosure, were in good agreement with the lake (cf. Figs. 1–3). To minimize the negative effects of the approach, the experiments lasted only for 2.5 weeks, a period assumed to be long enough even for the metazooplankton to respond to the manipulations. Because of limited resources no replicates within the experiments were used; instead, an intensive sampling procedure was used and several independent metabolic processes were measured. Although the results are difficult to interpret statistically, the experimental design with many subsamples will cover most of the variability within the microbial communities.

In accordance with the high N:P and C:P ratios in Lake Pääjärvi, autotrophic production of plankton was markedly stimulated by the addition of phosphate-phosphorus. The response was most evident in summer (Fig. 10), when primary production was also stimulated by the addition of humic water. The primary reason for this increase in primary production may not have been the HM addition itself but the phosphate-phosphorus that came along with the HM addition. In summer the biomass of zooplankton was highest and their grazing and excretion thus most intense (cf. Ejsmont-Karabin 1984). The added phosphorus enhanced primary production more in summer, during the rapid turnover of phosphorus, than in spring or in autumn.

Besides phosphorus, light is a factor strongly limiting primary productivity in Lake Pääjärvi, where light attenuation is rapid owing to high concentrations of coloured humic substances. As the HM addition simultaneously worsened the light conditions, stimulation of primary production by the extra phosphorus introduced along with HM was not as pronounced as expected. In summer, during thermal stratification, the euphotic zone is usually less than 30–40% of the epilimnion and 20% of the water mass of the lake (cf. Ilmavirta and Kotimaa 1974). The importance of light as a factor controlling primary productivity was also highlighted in winter experiments under ice, where phosphorus addition did not enhance primary production (Tulonen et al. 1994).

The addition of phosphorus caused a slight increase in bacterial production especially during the spring and summer experiments, indicating phosphorus limitation. However, a close relationship between primary production and bacterial production was still more clear (Fig. 11); maxima in bacterial production usually occurred simultaneously or just after the maxima in primary production (Figs. 1–3). This was consistent with the close coupling between algae and bacteria observed in late winter succession of plankton in Lake Pääjärvi (Tulonen et al. 1994) as well as in small forest lakes (Arvola and Kankaala 1989).

Usually bacterial cells have a higher affinity for inorganic phosphorus than algae, and therefore they are more capable of satisfying their phosphorus demands (Currie and Kalff 1984; Salonen et al. 1994). In spite of this, in some circumstances bacteria are not able to reach their physiological phosphorus saturation, and direct limitation of bacterial production by dissolved inorganic phosphorus has been detected in many lakes (Toolan et al. 1991; Coveney and Wetzel 1992; Bell et al. 1993). In general, the results from phosphatase activity measurements did not support this hypothesis as the activities were high only in the second half of the spring experiment, when phytoplankton biomass was maximal and water temperature increased rapidly. During that time competition for phosphorus seemed to be strongest (cf. Cotner and Wetzel 1992; Salonen et al. 1992*a*). However, phosphatase activity in summer was low in all enclosures as well as in the lake, and compared with the phosphatase activities in the polyhumic lake Mekkojärvi (Münster et al. 1992), the maximum values for Lake Pääjärvi were low (6–30%). In autumn, phytoplankton biomass and primary productivity were minimal and presumably the competition for phosphorus was less severe. However, inorganic nutrients may stimulate the degradation of

© 1996 NRC Canada

Table 4. Average ratios between primary production and community respiration in the various experiments.

	L	C	H	P	HP
Spring	0.394	0.386	0.419	0.453	0.492
Summer	0.383	0.409	0.445	0.581	0.511
Autumn	0.324	0.511	0.374	0.484	0.463

allochthonous organic matter by bacteria, and hence also improve the growth efficiency of bacteria (cf. Benner et. al. 1988; Tulonen et al. 1992). This possibility was supported by the observation that when extra phosphorus was added a slight increase in bacterial production was found in spring and summer. The growth efficiency of bacteria also depends on the C:N ratio of the substrate (Goldman et al. 1987; Kroer 1993), but nitrogen limitation in Lake Pääjärvi with a very high concentration of nitrate-nitrogen was highly improbable.

The close coupling between primary and bacterial production cannot be simply explained by phytoplankton exudates. By applying a very high bacterial growth efficiency of 50% (Lignell 1990; Sundh and Bell 1992; Kroer 1993), we calculated that only 45–80% of bacterial gross production in spring and summer and 15–43% in autumn could have been supported by exudates (cf. Kankaala et al. 1996). Autumn was the only period when a distinct response of bacteria to HM was observed. This might have resulted from the deficiency of easily degradable extracellular products at the time when autochthonous primary production was low. It is also possible that runoff water contained more labile organic compounds in autumn than in spring or in summer (cf. Ivarsson and Jansson 1994*a*).

Without phosphorus and (or) HM enrichments the ratio of bacterial production to primary production was on average 0.23 in spring and summer and 0.32 in autumn, values that are within the general range for aquatic ecosystems (Cole et al. 1988). In the enclosures with extra HM, the summer and autumn ratios were higher (0.30 and 0.59) than in the lake (0.19 and 0.27) or in the control (0.27 and 0.39), indicating a higher relative importance of allochthonous DOC as an energy and carbon source for bacteria (cf. Tranvik 1990; Tulonen et al. 1992; Kroer 1993). These values represent only the euphotic zone (0–3 m); in the whole water column of the lake (average depth 14 m) the bacterial production to primary production ratio is usually ca. 0.5 (T. Tulonen, unpublished data). Consistent with the pronounced heterotrophy, the community respiration of plankton was high in comparison with the measured primary production (primary production to respiration ratio (PP:R) varied between 0.3 and 0.6, Table 4). This agrees with the results of del Giorgio and Peters (1993, 1994) from 20 southern Quebec lakes, where plankton community respiration was 2–8 times greater than phytoplankton photosynthesis in all oligotrophic and mesotrophic lakes. Consistent with their observations that the imbalance of PP:R narrowed toward the more productive lakes, in our study the highest PP:R ratios were always recorded in those enclosures treated with phosphorus (with the exception of the control enclosure in autumn).

A reduction in turbulence and wave action could have promoted the sedimentation of small nonmotile particles in the enclosures. The sedimentation rate clearly increased in the enclosures with HM addition, but the enhanced primary and bacterial production did not increase the sedimentation rate. This was particularly true in summer in the enclosure with phosphorus enrichment, where primary and bacterial production were high, but the sedimentation rate of carbon was at the same level as in the control. The C:P ratios of the sedimented material decreased after the phosphorus additions, but only a relatively small amount of the added phosphorus was sedimented in the course of the experiments. The results indicate that during the experiments high amounts of organic matter were lost by respiration. Particularly in summer, when production of protozoa was high, the bulk of produced organic matter was lost in respiration of the microbial food chain (cf. Kankaala et al. 1996).

Only some algal taxa, mostly cryptophytes, responded distinctly to the additions of phosphorus and (or) HM. Cryptophytes are common in humic lakes (Arvola 1986; Arvola et al. 1990*a*) and are numerous also in Lake Pääjärvi (Ilmavirta and Kotimaa 1974). They are known to be heavily grazed by metazooplankton (Elser 1992), and they are also a high-quality food for zooplankton (Ahlgren et al. 1990; Santer 1994). Other species with a pronounced response to the addition of HM were *Katablepharis ovalis*, a colourless cryptophyte, and *Uroglena americana*, a mixotrophic chrysophyte (Bird and Kalf 1987; see also Jones 1993). The pooled data showed a statistically significant correlation ($P < 0.05$) between the biomass of bacteria and that of *Cryptomonas* spp. in three of the five treatments; the strongest correlations were found in enclosure P ($R^2 = 0.86$) and in the lake ($R^2 = 0.59$). Some other species (*Rhodomonas lens, Rhodomonas lacustris* v. *nannoplanctica, Cryptomonas* spp., *Chlamydocapsa planctonica,* and *Rhizosolenia longiseta*) responded to phosphorus addition but not to HM addition without phosphorus. From the listed taxa, at least the genus *Cryptomonas* is known to contain mixotrophic species (Antia 1980; Nygaard and Tobiasen 1993), although the carbon metabolism of the cells is mainly based on photosynthesis (Tranvik et al. 1989).

As expected, the impacts of phosphorus and HM enrichment on the higher trophic levels (protozoans and metazooplankton) were not as marked as on phyto- and bacterio-plankton. This is in accordance with other studies on nutrient enrichments (cf. O'Brien et al. 1992 for a review). In our study the lack of response could be partly due to the relatively short duration of the experiments. Only fast growing HNF showed higher biomasses in spring and in summer in the treatments with phosphorus and (or) HM enrichments. In contrast, metazooplankton with long developmental times did not respond strongly, especially when water temperature was low. The grazing impact by metazooplankton on protozoans (cf. Carrick et al. 1991; Arndt 1993) as well food competition and interference between cladocerans and rotifers (MacIsaac and Gilbert 1989, 1991) further complicates the interpretation of the results. Negative correlation between numbers of rotifers and cladocerans could be seen especially in spring and summer experiments. However, the highest biomasses of metazooplankton (especially cladocerans) were observed in summer in the HP treatment.

In conclusion, the data suggest that in this mesotrophic, humic lake the production of bacteria was stimulated more by substrates released by algae than directly by allochthonous HM or phosphorus, whereas phytoplankton growth was more clearly limited by phosphorus. Thus, the first hypothesis of this paper must be modified or even rejected. The results are in

© 1996 NRC Canada

contrast to those from small polyhumic lakes (Hessen et al. 1990; Salonen et al. 1992*b*), but in general agreement with the results of Søndergaard et al. (1988) from an oligotrophic Danish lake. On the other hand, inorganic nutrients evidently acted as regulators for the utilization of HM by bacteria, especially when phosphorus and HM were present in excess. This indicates that the second hypothesis of this paper might be accepted. The amount of carbon released by community respiration exceeded clearly the amount of carbon incorporated in primary production and lost by sedimentation, indicating severe losses of organic carbon and energy within the microbial food chain. Thus, the impacts of phosphorus and HM enrichment on the higher trophic levels were not as distinct as on phyto- and bacterio-plankton, a result not in good agreement with the third hypothesis of this study.

If the load of phosphorus and allochthonous organic matter in the boreal zone increases for some reason, the most evident short-term impact on lake metabolism and plankton food webs will take place during the warmest period of the year, when thermal stratification prevails in most humic lakes. If higher runoff and increased DOC inputs to the rivers and lakes take place in autumn (cf. Ivarsson and Jansson 1994*b*), the risk of oxygen depletion may become more acute under ice in many lakes because of enhanced bacterial production (Tulonen et al. 1994).

Acknowledgements

This study is a part of the Finnish Research Programme on Climate Change (SILMU) and was funded by the Academy of Finland. We thank R.I. Jones for comments and correcting the language of the manuscript.

References

Ahlgren, G., Lundstedt, L., Brett, M., and Forsberg, C. 1990. Lipid composition of some freshwater phytoplankton for cladocean zooplankters. J. Plankton Res. **12**: 809–818.

Antia, N.J. 1980. Nutritional physiology and biochemistry of marine cryptomonads and chrysomonads. *In* Biochemistry and physiology of protozoa. Vol. 3. 2nd ed. *Edited by* M. Lewandowsky and S.H. Huther. Academic Press, New York. pp. 67–115.

Arndt, H. 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates): a review. Hydrobiologia, **255–256**: 231–246.

Arvola, L. 1986. Spring phytoplankton of 54 small lakes in southern Finland. Hydrobiologia, **137**: 125–134.

Arvola, L. 1991. Recent trends in the water chemistry of lake Pääjärvi. *In* Lammi notes. Vol. 18. Lammi Biological Station, Helsinki. pp. 1–5.

Arvola, L., and Kankaala, P. 1989. Winter and spring variability in phyto- and bacterioplankton in lakes with different water colour. Aqua Fenn. **19**: 29–39.

Arvola, L., Metsälä, T.-R., Similä, A., and Rask, M. 1990*a*. Phyto- and zooplankton in relation to water pH and humic content in small lakes in southern Finland. Verh. Int. Ver. Theor. Angew. Limnol. **24**: 688–692.

Arvola, L., Salonen, K., and Rask, M. 1990*b*. Chemical budgets for a small dystrophic lake in southern Finland. Limnologica, **20**: 243–251.

Bell, R.,Vrede, K., Stensdotter-Blomberg, U., and Blomqvist, P. 1993. Stimulation of the microbial food web in an oligotrophic, slightly acidified lake. Limnol. Oceanogr. **38**: 1532–1538.

Benner, R., Lay, J., K'nees, E., and Hodson, R.E. 1988. Carbon conversion efficiency for bacterial growth on lignocellulose: implications for detritus based food webs. Limnol. Oceanogr. **33**: 1514–1526.

Bergström, I., Heinänen, A., and Salonen, K. 1988.Comparison of acridine orange, acriflavine and bisbenzimide stains for enumeration of bacteria in clear and humic waters. Appl. Environ. Microbiol. **51**: 664–667.

Bergström, S., and Carlsson, B. 1994. River runoff to the Baltic Sea: 1950–1990. Ambio, **33**: 280–287.

Bird, D.F., and Kalff, J. 1987. Algal phagotrophy: regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). Limnol. Oceanogr. **32**: 277–284.

Boer, M.M., Koster, E.A., and Lundberg, H. 1990. Greenhouse impact in Fennoscandia: preliminary findings of a European workshop on the effects of climatic change. Ambio, **19**: 2–10.

Carrick, H.J., Fahnenstiel, G.L., Stoermer, E.F., and Wetzel, R.G. 1991. The importance of zooplankton–protozoan couplings in Lake Michigan. Limnol. Oceanogr. **36**: 1335–1345.

Cole, J.J., Findlay, S., and Pace, M.L. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. **43**: 1–10.

Cotner, J.B., Jr., and Wetzel, R.G. 1992. Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacterioplankton. Limnol. Oceanogr. **37**: 232–243.

Coveney, M.F., and Wetzel, R.G. 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. Appl. Environ. Microbiol. **58**: 150–156.

Currie, D.J., and Kalff, J. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. **29**: 298–310.

Del Giorgio, P.A., and Peters, R.H. 1993. Balance between phytoplankton production and plankton respiration in lakes. Can. J. Fish. Aquat. Sci. **50**: 282–289.

Del Giorgio, P.A., and Peters, R.H. 1994. Patterns in planktonic P:R ratios in lakes: influence of lake trophy and dissolved organic carbon. Limnol. Oceanogr. **39**: 772–787.

Ejsmont-Karabin, J. 1984. Phosphorus and nitrogen excretion by lake zooplankton (rotifers and crustaceans) in relationship to individual body weights of the animals, ambient temperature and presence or absence of food. Ekol. Pol. **32**: 2–42.

Eloranta, P. 1978. Light penetration in different types of lakes in Central Finland. Holoarct. Ecol. **1**: 362–366.

Elser, J.J. 1992. Phytoplankton dynamics and the role of grazers in Castle Lake, California. Ecology, **73**: 887–902.

Forsberg, C. 1992. Will an increased greenhouse impact in Fennoscandia give rise to more humic and coloured lakes? Hydrobiologia, **229**: 51–58.

Geller, A. 1983. Degradability of dissolved organic lake water compounds in cultures of natural bacterial communities. Arch. Hydrobiol. **99**: 60–79.

Goldman, J.C., Caron, D.A., and Dennett, M.R. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr. **32**: 1239–1252.

Heikkinen, K. 1989. Organic carbon transport in an undisturbed boreal humic river in northern Finland. Arch. Hydrobiol. **117**: 1–19.

Hessen, D.O., Andersen, T., and Lyche, A. 1990. Carbon metabolism in a humic lake: pool sizes and cycling through zooplankton. Limnol. Oceanogr. **35**: 84–99.

Ilmavirta, K., and Kotimaa, A. 1974. Spatial and seasonal variations in phytoplanktonic primary production and biomass in the oligotrophic lake Pääjärvi, southern Finland. Ann. Bot. Fenn. **11**: 112–120.

Intergovernmental Panel on Climate Change. 1990. Climate change: the IPCC scientific assesment. *Edited by* J.T Houghton, G.J. Jenkins, and J.J. Ephraums. Cambridge University Press, Cambridge, U.K.

Ivarson, H., and Jansson, M. 1994*a*. Temporal variations in the

© 1996 NRC Canada

concentration and character of dissolved organic matter in a highly colored stream in the coastal zone of Northern Sweden. Arch. Hydrobiol. **132**: 45–55.

Ivarson, H., and Jansson, M. 1994*b*. Temporal variations of organic carbon in the River Öre, Northern Sweden. Verh. Int. Ver. Theor. Angew. Limnol. **25**: 1522–1525.

Jackson, T.A., and Hecky, R.E. 1980. Depression of primary production by humic matter in lake and reservoir waters of boreal forest zone. Can. J. Fish. Aquat. Sci. **37**: 2300–2317.

Jeffrey, S.W., and Hallegraeff, G.M. 1987. Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. Mar. Ecol. Prog. Ser. **35**: 293–304.

Jones, R.I. 1993. Mixotrophy in planktonic protists as a spectrum of nutritional strategies. Mar. Microb. Food Webs, **8**: 87–96.

Jones, R.I., and Arvola, L. 1984. Light penetration and some related characteristics in small forest lakes in southern Finland. Verh. Int. Ver. Theor. Angew. Limnol. **22**: 811–816.

Jones, R.I., Salonen, K., and DeHaan, H. 1988. Phosphorus transformations in the epilimnion of humic lakes: abiotic interactions between dissolved humic materials and phosphate. Freshwater Biol. **19**: 357–369.

Kämäri, J., Forsius, M., and Kauppi, L. 1990. Statistically based lake survey: a representative picture of nutrient status in Finland. Verh. Int. Ver. Theor. Angew. Limnol. **24**: 663–666.

Kankaala, P., Arvola, L., Tulonen, T., and Ojala, A. 1996. Carbon budget for the pelagic food web of the euphotic zone in a boreal lake (Lake Pääjärvi). Can. J. Fish. Aquat. Sci. **53**: 1663–1674.

Kauppi, L. 1984. The contribution of agricultural loading to eutrophication in Finnish lakes. Water Sci. Technol. **17**: 1133–1140.

Koroleff, F. 1979. Methods for the chemical analysis of seawater. (In Finnish.) *In* Meri. Vol. 7. Institute of Marine Research, Helsinki. pp. 1–60.

Kroer, N. 1993. Bacterial growth efficiency on natural dissolved organic matter. Limnol. Oceanogr. **38**: 1282–1290.

Latja, R., and Salonen, K. 1978. Carbon analysis for the determination of individual biomasses of planktonic animals. Verh. Int. Ver. Theor. Angew. Limnol. **20**: 2555–2560.

Lignell, R. 1990. Excretion of organic carbon by phytoplankton: its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. Mar. Ecol. Prog. Ser. **68**: 85–89.

MacIsaac, H.J., and Gilbert, J.J. 1989. Competition between rotifers and cladocerans of different body sizes. Oecologia, **81**: 295–301.

MacIsaac, H.J., and Gilbert, J.J. 1991. Discrimination between exploitative and interference competition between Cladocera and *Keratella cochlearis*. Ecology, **72**: 924–937.

McDowell, W.H., and Likens, G. 1988. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. Ecology, **58**: 177–195.

Meili, M. 1992. Sources, concentrations and characteristics of organic matter in softwater lakes and streams of the Swedish forest region. Hydrobiologia, **229**: 23–41.

Moran, M.A., and Hodson, R.E. 1990. Bacterial production on humic and nonhumic components of dissolved organic carbon. Limnol. Oceanogr. **35**: 1744–1756.

Murphy, J., and Riley, J.P. 1963. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, **27**: 31–36.

Münster, U., and Chróst, R.J. 1990. Origin, composition and microbial utilization of dissolved organic matter. *Edited by* J. Overbeck and R.J. Chróst. *In* Aquatic microbial ecology. New York. pp. 8–46.

Münster, U., Einiö, P., and Nurminen, J. 1989. Evaluation of the measurements of extracellular enzyme activities in a polyhumic lake by means of studies with 4-methyl-umbelliferyl-substrates. Arch. Hydrobiol. **115**: 321–337.

Münster, U., Einiö, P., Nurminen, J., and Overbeck, J. 1992. Extracellular enzymes in a polyhumic lake: important regulators in detritus processing. Hydrobiologia, **229**: 225–238.

Nygaard, K., and Tobiasen, A. 1993. Bacterivory in algae: a survival strategy during nutrient limitation. Limnol. Oceanogr. **38**: 273–279.

O'Brien, W.J., Hershey, A.E., Hobbie, J.E., Hullar, M.A., Kipphut, G.W., Miller, M.C., Moller, B., and Vestal, J.R. 1992. Control mechanisms of arctic lake ecosystems: a limnocorral experiment. Hydrobiologia, **240**: 143–188.

Prepas, E. 1978. Sugar-frosted *Daphnia*: an improved fixation technique for Cladocera. Limnol. Oceanogr. **23**: 557–559.

Putt, M., and Stoecker, D.K. 1989. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr. **34**: 1097–1103.

Rocha, O., and Duncan, A. 1985. Relationship between cell carbon and cell volume in freshwater algae species used in zooplankton studies. J. Plankton Res. **7**: 279–294.

Salonen, K. 1979. A versatile method for the rapid and accurate determination of carbon by high temperature combustion. Limnol. Oceanogr. **24**: 177–183.

Salonen, K. 1981*a*. The ecosystem of the oligotrophic Lake Pääjärvi. 2. Bacterioplankton. Verh. Int. Ver. Theor. Angew. Limnol. **21**: 448–453.

Salonen, K. 1981*b*. Rapid and precise determination of total inorganic carbon and some gases in aqueous solutions. Water Res. **15**: 403–406.

Salonen, K., and Arvola, L. 1986. Effect of added radiocarbon solution on primary production measurements. Water Res. **20**: 1041–1044.

Salonen, K., and Latja, R. 1988. Variation in the carbon content of two *Asplanchna* species. Hydrobiologia, **162**: 79–87.

Salonen, K., and Sarvala, J. 1985. Combination of freezing and aldehyde fixation, a superior method for biomass determination of aquatic invertebrates. Arch. Hydrobiol. **103**: 217–230.

Salonen, K., Kononen, K., and Arvola, L. 1983. Respiration of plankton in two small, polyhumic lakes. Hydrobiologia, **101**: 65–70.

Salonen, K., Arvola, L., Tulonen, T., Hammar, T., Metsälä, T.-R., Kankaala, P., and Münster, U. 1992*a*. Planktonic food chains of a highly humic lake. I. A mesocosm experiment during the spring primary production maximum. Hydrobiologia, **229**: 125–142.

Salonen, K., Kankaala, P., Tulonen, T. , Hammar, T., James, M., Metsälä, T.-R., and Arvola, L. 1992*b*. Planktonic food chains of a highly humic lake. II. A mesocosm experiment in summer during dominance of heterotrophic processes. Hydrobiologia, **229**: 143–157.

Salonen, K., Kairesalo, T., and Jones, R.I. (*Editors*). 1992*c*. Dissolved organic matter in lacustrine ecosystems: energy source and system regulator. Dev. Hydrobiol. Vol. 73.

Salonen, K., Jones, R.I., De Haan, H., and James, M. 1994. Radiotracer study of phosphorus uptake by plankton and redistribution in the water column of a small humic lake. Limnol. Oceanogr. **39**: 69–83.

Santer, B. 1994. Influences of food type and concentration on the development of *Eudiaptomus gracilis* and implications between calanoid and cyclopoid copepods. Arch. Hydrobiol. **131**: 141–159.

Schindler, D.W., Schmidt, R.V., and Reid, R. 1972. Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the 14C method. J. Fish. Res. Board Can. **29**: 1627–1631.

Schindler, D.W., Beaty, K.G., Fee, E.J., Cruikshank, D.R., DeBruyn, E.R., Findlay, D.L., Linsay, G.A., Shearer, J.A., Stainton, M.P., and Turner, M.A. 1990. Effects of climatic warming on lakes of the Central boreal forest. Science (Washington, D.C.), **250**: 967–970.

Schindler, D.W., Bayley, S.E., Curtis, P.J., Parker, B.R., Stainton, M.P., and Kelly, C.A. 1992. Natural and man-caused factors

© 1996 NRC Canada

affecting the abundance and cycling of dissolved organic substances in Precambrian Shield lakes. Hydrobiologia, **229**: 1–21.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14**: 799–801.

Søndergaard, M., Riemann, B., Jensen, L.M., Jørgensen, N.O.G., Bjørnsen, P.K., Olesen, M., Larsen, J.B., Geertz-Hensen, O., Hansen, J., Christoffersen, K., Jespersen, A.-M., Andersen, F., and Bosselmann, S. 1988. Pelagic food web processes in an oligotrophic lake. Hydrobiologia, **164**: 271–286.

Steeman Nielsen, E. 1952. The use of radioactive carbon (C^{14}) for measuring organic production in the sea. J. Cons. Cons. Int. Explor. Mer, **18**: 117–140.

Sundh, I., and Bell, R.T. 1992. Extracellular dissolved organic carbon released from phytoplankton as a source of carbon for heterotrophic bacteria in lakes of different humic content. Hydrobiologia, **229**: 93–106.

Toolan, T., Wehr, J.D., and Findlay, S. 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. Appl. Environ. Microbiol. **57**: 2074–2078.

Tranvik, L. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb. Ecol. **16**: 311–322

Tranvik, L. 1990. Bacterioplankton growth on fractions of dissolved organic carbon of different molecular weights from humic and clear waters. Appl. Environ. Microbiol. **56**: 1672–1677.

Tranvik, L.J., Porter, K.M., and Sieburth, J.M. 1989. Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. Oecologia, **78**: 473–476.

Tulonen, T. 1993. Bacterial production in a mesohumic lake estimated from (^{14}C)-leucine incorporation rate. Microb. Ecol. **26**: 201–217.

Tulonen, T., Salonen, K., and Arvola, L. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. Hydrobiologia, **229**: 239–252.

Tulonen, T., Kankaala, P., Ojala, A., and Arvola, L. 1994. Factors controlling production of phytoplankton and bacteria under ice in a humic, boreal lake. J. Plankton Res. **16**: 1411–1432.

Utermöhl, H. 1958. Zur Vervollkommung der quantitativen Phytoplanktonmethodik. Mitt. Int. Ver. Theor. Angew. Limnol. **9**: 1–38.

Verity, P.G., Robertson, C.Y., Tronzo, C.R., Andrews, M.G., Nelson, J.R., and Sieracki, M.E. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. **37**: 1434–1446.

Wetzel, R. G. 1983. Limnology. 2nd ed. Saunders, Philadelphia, Pa.

Wood, E.D., Armstrong, F.A.J., and Richards, F.A. 1967. Determination of nitrate in sea water by cadmium copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. **47**: 23–31.

© 1996 NRC Canada

Carbon budget for the pelagic food web of the euphotic zone in a boreal lake (Lake Pääjärvi)

Paula Kankaala, Lauri Arvola, Tiina Tulonen, and Anne Ojala

Abstract: A carbon budget for the pelagic euphotic zone of a mesohumic, boreal lake was constructed, on the basis of enclosure experiments, in which effects of pulses of humic matter and phosphorus were studied. Although production of phytoplankton and bacteria was enchanced by additions of phosphorus, and of phosphorus and humic matter together, the effects on zooplankton were less clear. The modest response of metazooplankton was partly due to the short time of the experiments, but it was also due to loss of carbon through respiration in several steps of the microbial food chain. Protozoans had key roles as grazers in the system; heterotrophic nanoflagellates were the most important grazers of bacteria, whereas ciliates were more important algal grazers than metazooplankton. Indirect evidence suggested that ciliates formed a considerable proportion of the food of metazooplankton. The amount of carbon released through respiration exceeded the sum of that fixed by primary producers and lost through sedimentation. If the climate change in the boreal zone increases loading of nutrients and humic matter into lakes, this imbalance between respiration and fixation of carbon can be expected to increase.

Résumé : Nous avons établi le bilan du carbone pour la zone euphotique pélagique d'un lac boréal mésohumique, à l'aide d'expériences en enclos dans lesquelles nous avons étudié les effets de poussées de matière humique et de phosphore. Bien que la production phytoplanctonique et bactérienne ait été accrue par l'ajout de phosphore, et de phosphore et de matière humique ensemble, les effets sur le zooplancton ont été moins évidents. La réponse modeste du métazooplancton était due en partie à la courte durée des expériences, mais aussi à la perte de carbone par la respiration à plusieurs étapes de la chaîne trophique microbienne. Les protozoaires jouaient un rôle clé en tant que brouteurs dans le système; les nanoflagellés hétérotrophes étaient les plus importants brouteurs de bactéries, tandis que les ciliés étaient des brouteurs d'algues plus importants que le métazooplancton. Des données indirectes permettent de penser que les ciliés formaient une proportion considérable de la nourriture du métazooplancton. La quantité de carbone libéré par la respiration dépassait la somme de celui qui était fixé par les producteurs primaires et perdu par la sédimentation.
[Traduit par la Rédaction]

Introduction

Productivity in clear-water lakes and oceans is based on autotrophic fixation of carbon by phytoplankton and rooted macrophytes, and the level of primary production determines the productivity of higher trophic levels (e.g., Makarewicz and Likens 1979; Cole et al. 1988). A close coupling of algae and bacteria owing to the dependence of bacteria on algal exudates is well documented in marine and lake ecosystems (e.g., Larsson and Hagström 1982; Pomeroy and Wiebe 1988; Søndergaard et al. 1988; Vadstein et al. 1989). In humic lakes there is a large pool of dissolved organic carbon of allochthonous origin (cf. Arvola et al. 1990; Hessen et al. 1990; Salonen et al. 1992*a*, 1992*b*). Although this humic matter (HM) is refractory, it is potentially an important source of energy in the ecosystem (Salonen and Hammar 1986; Meili 1993). The energy is transmitted to higher trophic levels via bacteria, either directly or through the microbial food chain (Salonen et al. 1992*a*, 1922*b*; Tranvik and Höfle 1987; Tranvik 1988, 1990). The low ratios of primary production to community respiration indicate the importance of allochthonous carbon in these systems (Salonen et al. 1983; Jones and Salonen 1985; del Giorgio and Peters 1993, 1994).

Increased loadings of HM and phosphorus, which are considered possible consequences of the increasing temperature and precipitation in the European boreal zone associated with climate change (Boer et al. 1990; Intergovernmental Panel on Climate Change 1990), were studied in the pelagic system of Lake Pääjärvi. This is a typical mesohumic lake of the boreal zone, where the annual input of allochthonous organic carbon exceeds that fixed by phytoplankton and littoral vegetation (Sarvala et al. 1981). In this paper we describe the carbon budget and carbon flow through the food web on the basis of enclosure experiments and measurements carried out in the euphotic zone of the lake reported in Arvola et al. (1996).

Methods

The effects of pulses of HM and phosphorus were studied three times during the ice-free period of 1991 in enclosure (volume 8.5 m^3) experiments lasting for 16–17 days. Humic water from the catchment was added to the first enclosure (H), corresponding to about 5% of the total volume and 5–27% of DOC. In the second enclosure (P) the concentration of phosphate was increased ca. 10-fold above that in lake (from 1–2 to 15–20 μg PO_4-P·L^{-1}). Both humic water and PO_4-P were added to the third enclosure (HP). The fourth enclosure (C) did not receive any enrichments and served as a control (Arvola et al. 1996). All measurements made in the enclosures were also made in the lake (L) at equivalent depths (0–3 m).

The loss rate (LR) of algal biomass from grazing, sedimentation,

Received May 4, 1995. Accepted February 15, 1996.
J12905

P. Kankaala,[1] L. Arvola, T. Tulonen, and A. Ojala.
University of Helsinki, Lammi Biological Station, FIN–16900 Lammi, Finland.

[1] Author to whom all correspondence should be addressed.
e-mail: paula.kankaala@helsinki.fi

© 1996 NRC Canada

and cell lysis was estimated as the difference between the measured primary production (excluding the extracellularly released proportion) (Pp) and apparent primary production (Pa), according to Garnier and Mourelatos (1991): $LR = Pp - Pa$, and $Pa = (Bt_2 - Bt_1)/(t_2 - t_1)$, where Pp is the daily primary production measured with the ^{14}C technique (averages for consecutive sampling days t_1 and t_2) and Bt_1 and Bt_2 are the phytoplankton biomasses on days t_1 and t_2, respectively. The loss rate of bacterial biomass was calculated with the same method as the difference between the measured ([^{14}C]leucine) and apparent bacterial production.

Protozoan grazing was estimated with the multiple regression model of Peters (1994): $IR = 0.002\ VPY^{-0.344}\ VPD^{0.477}\ CPY^{0.489}\ CPD^{-0.270}\ 10^{(0.033\ T)}$, where IR is the ingestion rate (number of prey per predator per hour), VPY is the prey cell volume (cubic micrometres), VPD is the predator cell volume (cubic micrometres), CPY is the number of prey per millilitre, CPD is the number of predators per millilitre, and T is the ambient water temperature (°C). Heterotrophic nanoflagellates (HNF) were assumed to feed only on bacteria, although small colloidal dissolved organic matter (DOM) and viruslike particles may sometimes be a supplementary food source for HNF (Tranvik 1994). Ciliates were assumed to feed on bacteria, algae, and HNF. For the grazing calculations, algae and HNF were grouped into size-classes of $<10^2$ and 10^2–10^3 μm^3, excluding all colony-forming species and those with spiny and bristly morphology (e.g., most diatoms and *Mallomonas* sp.). The ciliates with volume $>10^3$ μm^3 were assumed to feed on particles of both size-classes, and those $<10^3$ μm^3 were assumed to feed on particles smaller in volume than themselves. The gross growth efficiency of protozoans was assumed to be 40% (Fenchel 1987), hence net production = 0.4 IR. The net production of HNF (including craspedomonads and *Katablepharis ovalis*) was also estimated by another method: for each experiment the maximum biomass growth rate (k_{max}) was estimated and this was multiplied by the average biomass of successive samplings according to Lignell et al. (1993).

Metazooplankton community grazing was determined in spring (three experiments) and in summer (two experiments). Pooled (0–3 m) samples were taken from the lake and from each enclosure with a 2-L tube sampler. At the beginning of the spring experiments the samples were concentrated ca. 2-fold with a 50-μm mesh immediately after sampling and transported to the laboratory where the samples were divided into 2–3 subsamples. One subsample (in a 1-L glass bottle) was filtered on a 70-μm mesh net and the animals were preserved with formaldehyde for later identification and counting. The other subsamples were placed in a water bath adjusted to the temperature (±0.5°C) in the lake at 0–3 m. Irradiation was 100–110 μmol·m^{-2}·s^{-1}. *Chlamydomonas* sp. (diameter 7 μm, length 12 μm, volume ca. 300 μm^3) was used as a food label. Algae were cultured for 4–5 days in 20% Jaworski's medium (Thompson et al. 1988) with 6–6.5 kBq $Na_2{}^{14}CO3$ added, and prior to the experiments excess inorganic ^{14}C was removed with acidification, bubbling, and neutralization (Holtby and Knoechel 1981). Grazing experiments were conducted after bottles had been incubated for 2 h in the water bath. *Chlamydomonas* sp. was added at a density less than 10% of that of natural phytoplankton and the bottles were shaken gently. Experiments lasted 15 min in spring and 10 min in summer. Blanks were prepared according to the same procedure except that animals were killed with formaldehyde (final concentration 0.8%) ca. 15 min before the labelled algae were added. Incubations were terminated by filtering the animals on a 70-μm net, whereupon they were narcotized with carbonated water and washed onto small sheets of gauze, which were placed into liquid scintillation vials at ±0°C. Radioactivity of animals and algae was determined as described in Kankaala (1988). In the present experiments zooplankton biomass (ZB, mg C·m^{-3}) and temperature (7.3–20.7°C) explained 72% of the variation in community clearance rate (CR, L·m^{-3}·day^{-1}). Thus, the model $\ln CR = 0.997 + 0.458 \ln ZB + 0.100\ T$ ($r^2 = 0.72$, $n = 22$) was applied to estimate the community clearance rates of metazooplankton. The feeding rate of metazooplankton was estimated by multiplying the community clearance rate by the biomass of algae <25 μm. HNF was assumed to be grazed at the same rate as algae. The proportion of bacteria as a food for cladocerans was estimated from the equation $\ln CR = 2.176 \ln L + 0.708$, where CR is the clearance rate (millilitres per individual in each day) and L is the length of the cladoceran in millimetres (Knoechel and Holtby 1986*b*). For rotifers, bacteria were proven to be an insignificant food source (the daily clearance rate on bacteria was assumed to be 5 μL/individual according to results of Sanders et al. 1989). Because *Eudiaptomus gracilis*, the dominant copepod in Lake Pääjärvi, did not graze on [^{14}C]leucine-labelled bacteria (P. Kankaala, unpublished data), the copepods in general were assumed to be incapable of feeding on bacteria in Lake Pääjärvi.

The production of metazooplankton was calculated with the growth increment method according to Winberg et al. (1971). Generation times of rotifers were taken from Galkovskaya (in Winberg 1971) and Makarewicz and Likens (1979). The development times of the dominant crustacean zooplankton taxa at the prevailing temperatures were estimated from the equations of Bottrell et al. (1976), Vijverberg (1980), and Kankaala and Wulff (1981). For the monocyclic *Limnocalanus macrurus* the data of Latja (R.L. Eteläkatu, 16 A 4, FIN–80110 Joensuu, Finland, unpublished data) from Lake Pääjärvi were applied. According to these data the development takes ca. 150 days in winter for naupliar stages and ca. 30 days in May–June for copepodites. The growth increment for each developmental stage was obtained from the individual carbon content analyses (see Arvola et al. 1996). The somatic growth increment for each adult cladoceran instar was assumed to be equal, and for the estimation of total growth the carbon mass of eggs and embryos was added to this.

Carbon flow budgets were established for enclosures C, P, and HP. Average values of biomass and production in the 3-m deep water column (mg C·m^{-3}·day^{-1}) at different trophic levels (algae, bacteria, HNF, ciliates, and metazooplankton) were applied. Carbon flow was described as $P = C - R$, where P is net production (from Arvola et al. 1996 and Tables 1–3), C is food consumption, and R is respiration. This was a simplification, as the proportion excreted and lost as feces from zooplankton was not estimated. However, this was at least partly included in the measurements of phytoplankton exudation that were determined as the amount of fixed ^{14}C in the <1-μm fraction after a 24-h incubation in bottles containing the phytozooplankton communities (cf. Arvola et al. 1996). All ciliates, as well as metazooplankton, were assumed to be on the same trophic level as there was no reliable criterion to separate herbivores from predators.

Results

In Lake Pääjärvi the majority of organic carbon is in the form of dissolved organic carbon (concentration about 10 mg C·L^{-1}, see Arvola et al. 1996), and in summer 1991 the ratio of dissolved to particulate organic carbon (DOC:POC) varied between 44 and 60 (Fig. 1). In the enclosures the ratio was lower, especially in summer in H, P, and HP. In autumn, when the DOC:POC ratios were highest, the proportion of particulate detritus (POC remaining after the subtraction of biomass of phytoplankton, bacteria, protozoa, and metazoa) was also maximal both in the lake (50%) and in the enclosures (52–57%). In spring and summer the proportion was lower, especially in the P enclosure (<10%). In summer the addition of HM resulted in an increase in detrital POC (only control and HP enlosures presented as examples in Fig. 2). In spring chlorophyll *a* was positively correlated to POC ($r^2 = 0.87$, $p < 0.001$, df = 28), and during the phytoplankton maximum the ratio of POC to chlorophyll *a* varied between 24 and 35 (average 30.6 ± 4.7). In summer and in autumn the correlation was not significant.

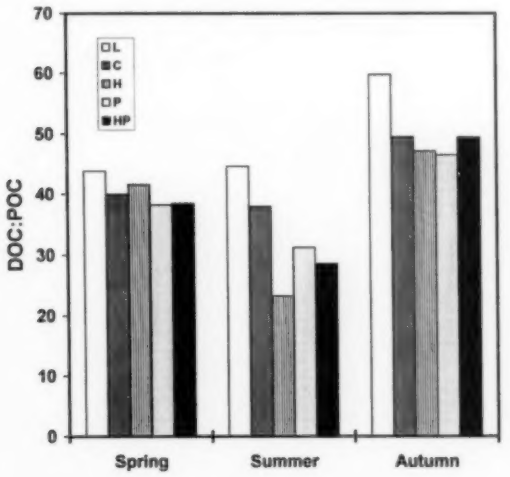
© 1996 NRC Canada

Table 1. Average ingestion rates of heterotrophic nanoflagellates on bacteria (mg C·m^{-3}·day^{-1}) and of ciliates, with percentages (%) of different sources in the food of ciliates.

	L	C	H	P	HP
Spring					
Ingestion rate					
HNF	4.5	5.7	6.8	10.8	7.3
	(3.0–7.4)	(2.2–13.3)	(4.3–12.6)	(3.3–27.1)	(2.9–14.8)
Ciliates	25.9	17.3	12.9	22.8	19.6
	(6.1–63.9)	(0.7–41.9)	(0.8–19.9)	(0.1–70.2)	(8.8–49.5)
Percentage					
Algae	62	54	45	50	51
HNF	23	28	34	35	30
Bacteria	15	18	21	15	19
Summer					
Ingestion rate					
HNF	8.9	12.4	18.3	15.8	16.6
	(2.4–15.6)	(4.2–19.3)	(11.4–26.0)	(9.9–23.9)	(7.9–28.2)
Ciliates	26.8	44.4	52.3	61.1	38.4
	(8.0–74.3)	(12.7–113.5)	(25.7–101.9)	(18.9–119.1)	(9.9–114.9)
Percentage					
Algae	67	65	59	70	62
HNF	16	18	24	16	22
Bacteria	17	17	17	14	16
Autumn					
Ingestion rate					
HNF	2.4	1.7	3.0	2.2	2.3
	(1.4–4.2)	(1.4–2.2)	(1.3–4.9)	(1.7–3.0)	(1.8–3.5)
Ciliates	5.6	7.6	7.9	6.7	8.2
	(0.5–16.4)	(2.5–14.6)	(4.0–14.6)	(2.6–10.9)	(1.2–14.9)
Percentage					
Algae	61	62	58	65	63
HNF	18	18	20	15	17
Bacteria	23	20	22	20	20

Note: Values in parentheses are ranges.

Fig. 1. Average ratio between dissolved and particulate organic carbon (DOC:POC) in Lake Pääjärvi (L) and in the enclosures (see Methods for symbols).



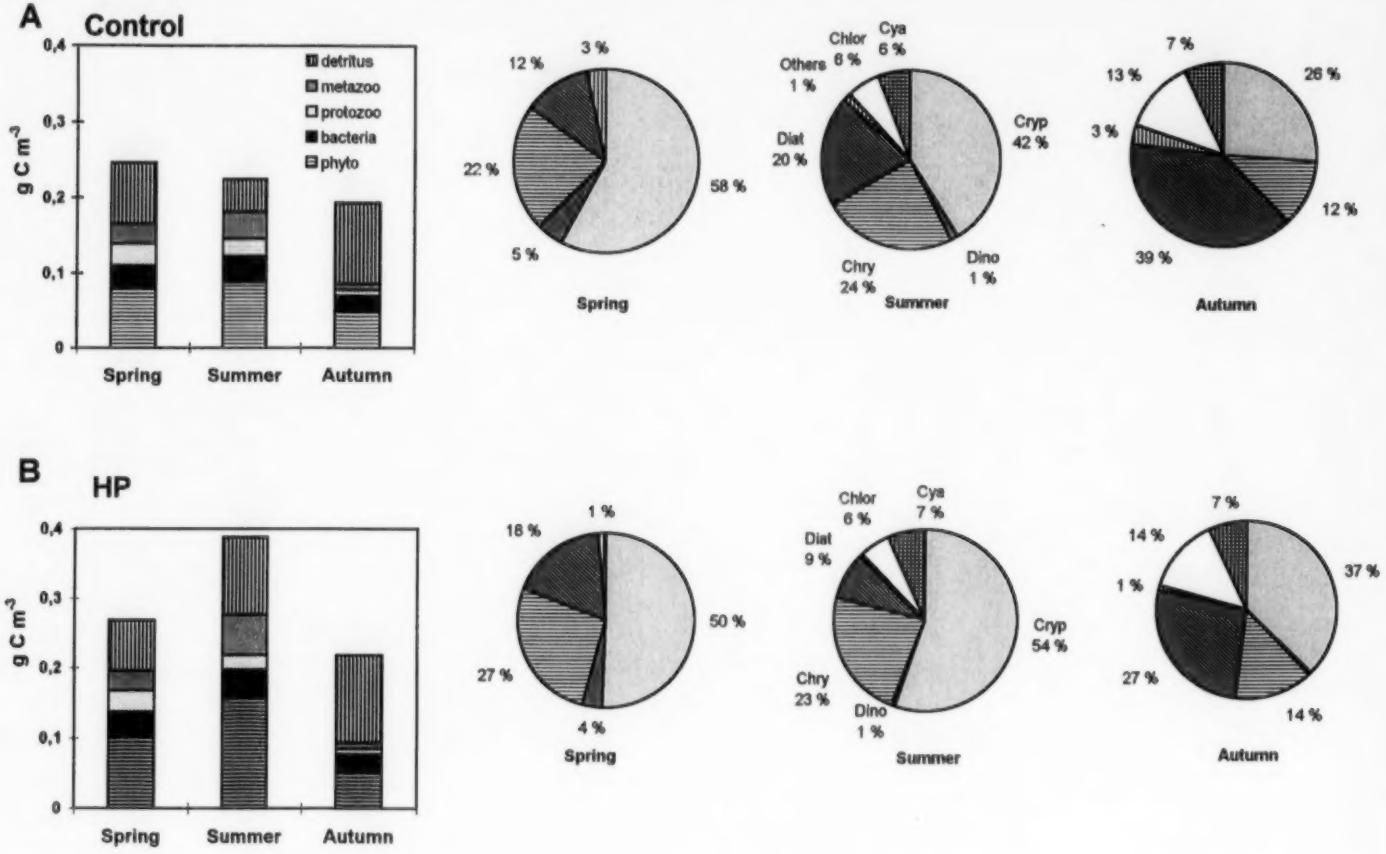
About half the total plankton biomass consisted of phytoplankton. Although the enrichment with phosphorus enchanced primary production and was also reflected in phytoplankton biomasses, the changes in the structure of the phytoplankton community were small (see Arvola et al. 1996). In spring and summer, cryptophytes formed 40–56% of the phytoplankton biomass, but in autumn their proportion was lower (Fig. 2). Chrysophytes and diatoms were important in all seasons and chlorophytes were important in autumn. Cyanophytes formed only 5–9% of the biomass in summer and autumn.

The estimates of grazing rates by protozoans and metazoan plankton (Tables 1–2) indicate that HNF was the group grazing most intensively on bacteria, whereas ciliates were more important algal grazers than metazooplankton. The clearance rates of the metazooplankton community were low, ranging from 5 to 60 L·m^{-3}·day^{-1} in spring, from 61 to 181 L·m^{-3}·day^{-1} in summer, and from 10 to 38 L·m^{-3}·day^{-1} in autumn. This means that at most 17–18% of the water volume was cleared daily.

The k_{max} of HNF varied between 0.3 and 0.6 day^{-1}. The production estimates based on k_{max} values were quite close to those obtained from grazing estimates (Table 3), which suggests that the estimates are of the right order of magnitude. In spring and summer the production of HNF was highest in the

© 1996 NRC Canada

Fig. 2. Average concentration of POC consisting of phytoplankton, bacteria, protozoa, metazoa, and particulate detritus in control (A) and HP enclosures (B). The average percent composition of phytoplankton is also given. Cya, Cyanophyta; Cryp, Cryptophyta; Dino, Dinophyta; Diat, Diatomophyta; Chlor, Chlorophyta.



© 1996 NRC Canada

Table 2. Average clearance rates (CR, $L \cdot m^{-3} \cdot day^{-1}$, averages and ranges) of the metazooplankton community on algae, along with ingestion rates (IR, mg $C \cdot m^{-3} \cdot day^{-1}$) and percentages of different food sources.

	L	C	H	P	HP
Spring					
CR	19.6	29.9	30.5	36.2	32.4
	(5.6–43.4)	(5.1–56.4)	(6.4–60.1)	(6.0–57.8)	(7.8–58.0)
IR	1.3	2.2	1.9	3.7	2.4
	(0.3–3.0)	(0.2–5.8)	(0.2–5.5)	(0.2–15.2)	(0.4–14.9)
Percentage					
Algae	87	85	82	81	83
HNF	11	12	17	18	14
Bacteria	2	3	3	1	3
Summer					
CR	115.5	100.8	95.2	118.7	137.2
	(101.9–163.0)	(68.0–138.8)	(60.7–136.9)	(96.4–180.9)	(96.0–168.4)
IR	8.0	6.5	11.2	16.1	18.3
	(2.1–17.0)	(3.7–11.0)	(6.5–22.5)	(6.5–35.0)	(8.5–29.8)
Percentage					
Algae	88	82	85	89	86
HNF	8	13	12	8	9
Bacteria	4	5	3	3	5
Autumn					
CR	18.8	17.4	21.5	16.6	19.1
	(14.0–27.7)	(11.4–27.4)	(10.4–38.1)	(12.5–21.7)	(9.3–30.8)
IR	0.4	0.4	0.8	0.7	0.7
	(0.1–1.2)	(0.1–0.8)	(0.2–1.6)	(0.1–1.1)	(0.4–1.2)
Percentage					
Algae	88	91	91	94	93
HNF	12	9	9	6	7
Bacteria	0	0	0	0	0

Note: Values in parentheses are ranges. IR values were obtained by multiplying CR by the combined biomass of algae and heterotrophic nanoflagellates <25 μm and then adding the IR on bacteria (estimated according to Knoechel and Holtby 1986*b*).

enclosures with phosphorus and (or) HM enrichments. Ciliates were responsible for the bulk of the protozoan production (Tables 4–6) and their production always exceeded that of metazooplankton, the difference being highest in autumn (9- to 16-fold) and lowest in summer (2- to 5-fold). The highest productivity for protozoan as well as for metazoan plankton was found in summer (14–31 and 6–9 mg $C \cdot m^{-3} \cdot day^{-1}$, respectively). Within the metazoan community, copepods had the highest productivity. The contribution of rotifers to metazoan production was significant in spring and in autumn, whereas the production of cladocerans was high only in summer and in the control enclosure in spring.

In the schematic carbon flows presented in Figs. 3–5, the production and biomass values (average values in milligrams of carbon per cubic metre for each experiment) come from Tables 4–6. The flux from phytoplankton to the DOC pool is the exuded proportion of the primary production. The gross growth efficiency of bacteria was assumed to be 30% (see Discussion), and the flux from DOC to bacteria was thus estimated by multiplying the bacterial production by a factor 3.3. The fluxes into HNF, ciliates, and metazoan plankton are based on grazing estimates (Tables 1–2).

The extracellular products of phytoplankton were highest in summer, when the daily exudation was about 0.2% of the average DOC pool. In spring about one third of the carbon demand of bacteria was satisfied by algal exudates both in the lake and in the enclosures. The same was true in the enclosures in summer, but then in the lake the DOC exuded by phytoplankton corresponded to about half of the bacterial demand. In autumn only 7–18% of the bacterial production in the enclosures and 30% in the lake was supported by algal exudates.

Table 3. Production of heterotrophic nanoflagellates (daily averages, mg $C \cdot m^{-3} \cdot day^{-1}$).

	L	C	H	P	HP
Spring					
Method A	2.7	2.7	3.0	5.3	3.9
Method B	1.8	2.3	2.7	4.3	2.9
Summer					
Method A	2.4	2.4	6.7	4.4	5.2
Method B	3.6	4.9	7.3	6.3	6.6
Autumn					
Method A	2.2	0.5	0.6	0.4	0.3
Method B	1.0	0.7	1.2	0.9	0.9

Note: Daily average production rates for different experiments were estimated by the maximum growth rate method (method A) and by grazing of heterotrophic nanoflagellates on bacteria according to Peters (1994) (method B), assuming 40% gross growth efficiency (see Methods for further details).

© 1996 NRC Canada

Table 4. Carbon budget variables for the lake and the enclosures averaged for the spring experiment.

	L	C	H	P	HP
Phytoplankton					
Production	32	34	33	40	42
Exudates	9	9	7	9	11
Biomass	86	78	71	136	98
Loss rate	25	28	28	20	33
P:B	0.372	0.436	0.465	0.294	0.429
Bacteria					
Production	8	7	7	9	10
Biomass	30	30	31	31	36
Loss rate	7	7	6	9	10
P:B	0.267	0.233	0.226	0.290	0.277
Protozoa					
Production	12	9	8	13	11
% HNF	5	25	35	32	27
% Ciliata	85	75	65	68	73
Biomass	39	32	28	38	33
P:B	0.308	0.281	0.286	0.342	0.333
Metazooplankton					
Production	0.7	1.9	1.9	1.6	2.1
% Rotatoria	38	23	38	42	32
% Cladocera	17	38	12	10	10
% Copepoda	45	39	50	48	58
Biomass	9	26	26	21	28
P:B	0.078	0.073	0.073	0.076	0.075
Community respiration	82	88	78	87	85
Sedimentation	23	nd	17	17	20

Note: Production, respiration, and sedimentation rates are in milligrams per cubic metre per day and biomasses are in milligrams of carbon per cubic metre (0–3 m). *P:B*, ratio of production to biomass; nd, no data.

Table 5. Carbon budget variables for the lake and the enclosures during the summer experiment (see Table 4 for further details).

	L	C	H	P	HP
Phytoplankton					
Production	46	47	55	72	74
Exudates	14	15	15	23	24
Biomass	78	85	118	161	152
Loss rate	31	30	46	57	52
P:B	0.590	0.553	0.466	0.447	0.487
Bacteria					
Production	9	13	17	20	21
Biomass	31	35	39	41	38
Loss rate	10	14	18	21	21
P:B	0.290	0.371	0.436	0.487	0.553
Protozoa					
Production	14	23	28	31	22
% HNF	25	22	26	21	30
% Ciliata	75	78	74	79	70
Biomass	15	24	29	24	26
P:B	0.933	0.958	0.966	1.192	0.846
Metazooplankton					
Production	7	7	6	7	9
% Rotatoria	5	4	5	12	7
% Cladocera	25	31	29	33	51
% Copepoda	70	65	66	55	43
Biomass	49	36	33	52	74
P:B	0.143	0.194	0.182	0.135	0.122
Community respiration	121	115	123	125	144
Sedimentation	29	19	nd	19	46

The production and consumption of bacteria were evidently in balance, as loss rates roughly equalled the production rates (Tables 4–6). In spring and summer, the estimated grazing pressure by HNF and ciliates on bacteria exceeded the bacterial production, but considering the high daily variation in these values (cf. Table 1 and Arvola et al. 1996), the estimated grazing rates are probably of the right order of magnitude. Although HNF were the most important grazers of bacteria, ciliates were also important, especially in autumn. The direct consumption of bacteria by metazooplankton (only cladocerans) was negligible (maximum 4% in summer in the HP enclosure). The grazing on HNF by ciliates and to a lesser extent by metazooplankton also exceeded the estimated production of HNF in spring and summer.

Ciliates were always the most important consumers of algae. In spring, the ciliates grazed 18–30% of the average primary production in the enclosures (maximal in the P enclosure) and 50% in the lake. In summer, ciliates were estimated to consume up to 62% of the primary production in the P and control enclosures, 20–32% in the H and HP enclosures, and 39% in the lake. In autumn, the ciliate grazing was 31–38% of the primary production in the control, H, and HP enclosures, 19% in the P enclosure, and 28% in the lake. Metazoan grazing consumed only 5–8% of the primary production in spring. In summer the impact was slightly higher, when the metazoan plankton consumed 11% of the primary production in the control enclosure, 17–22% in the H, P, and HP enclosures, and 15% in the lake. In autumn only 3–6% of the primary production was consumed by metazoan plankton.

The proportion of algal biomass lost through sedimentation or autolysis was estimated by subtracting the consumption of algae by ciliates and metazoan plankton from the total loss rates (Tables 4–5). Because samples were taken every 3rd day, some peaks of primary production may have been missed during the experiments. For example, in spring in the P enclosure, the phytoplankton biomass almost doubled between days 9 and 12 (data not shown), but the primary production on day 12 was about one third of that measured on day 9 (cf. Arvola et al. 1996). In the lake, the loss rate estimates were also affected by shifting water masses. However, taking these limitations into consideration, the proportion of the algal biomass sedimented or autolysed was higher in spring (24% of the primary production in the lake, 13% of that in the P enclosure, and 50–64% of that in the control, H, and HP enclosures) than in summer. In summer, grazing by ciliates and metazoan plankton slightly exceeded the algal loss rate in the control enclosure, indicating that either grazing was overestimated or the loss rate was underestimated. In the P enclosure the grazing by zooplankton equalled the algal loss rate, and in the lake and H and HP enclosures only 10–16% of the primary production was sedimented or autolysed. In autumn this proportion was again higher; 60 and 27–49% of the primary production was lost in

© 1996 NRC Canada

this way in the lake and in the enclosures, respectively. The stable sedimentation rate of carbon in the enclosures with increased primary production supports the conclusion that most of the produced algal material was grazed, especially in spring and summer. The sedimentation rate increased only in those enclosures where HM was added and in the lake as the bottom material was resuspending (cf. Arvola et al. 1996).

The amount of carbon released through community respiration was always higher than that fixed by primary production or lost through sedimentation. The ratio of carbon lost via respiration to that lost through sedimentation was 3.2–6.4 in spring and summer and 1.2–2.7 in autumn (Tables 4–6).

Discussion

Moderate enrichment with HM and phosphorus enhanced productivity of algae and bacteria especially in summer (Arvola et al. 1996), but the effects further in the food chain were less obvious. However, even in experiments with high nutrient loading lasting for several years, the responses of metazooplankton were modest or the productivity increased only after several experimental growing seasons (see O'Brien et al. 1992 for a review). In our experiments, of the carbon fixed by primary producers only 10–15% in summer and 2–6% in spring and autumn was transferred as net production of metazooplankton. Most of the material produced was consumed and respired in several steps of the microbial food chain so that metazoan plankton were not greatly affected.

Carbon budgets are sensitive to conversion factors and growth efficiencies applied to different compartments. The gross growth efficiency of bacteria has been shown to range from <10 to >60% (cf. Pomeroy and Wiebe 1988; Coffin et al. 1993; Kroer 1993). The efficiencies are highest when fresh phytoplankton exudates or macrophyte leachates are available as a substrate for bacteria (Findlay et al. 1986; Coffin et al 1993). In humic waters the gross growth efficiency of bacteria varies from 20 to 31% (Meyer 1987; Tranvik and Höfle 1987; Tranvik 1988; Tulonen et al. 1992) and is about 30% for bacteria utilizing lignocellulose from macrophyte detritus (Benner et al. 1988). In Lake Pääjärvi the gross growth efficiency of bacteria was assumed to be 30% without any seasonal variation, although we are aware that such variation has been observed in several studies (e.g., Kristiansen et al. 1992; Kroer 1993) The close coupling between primary and bacterial production in Lake Pääjärvi (Tulonen 1993; Arvola et al. 1996), a phenomenon typical of aquatic ecosystems (e.g., Cole et al. 1988; Søndergaard et al. 1988; Simon and Tilzer 1987), suggests a high gross growth efficiency for bacteria (cf. Sundh and Bell 1992; Coffin 1993). However, even by applying a gross growth efficiency of 50% in calculations, bacterial carbon demands could not have been entirely met by phytoplankton exudates. Moreover, the ratio between net primary production and community respiration (PP:R) in Lake Pääjärvi indicates that some extra carbon sources besides autochthonously fixed carbon are important in the food web. In humic lakes the most likely explanation for the low PP:R ratio is that allochthonous DOC is used by bacteria with low efficiency, and primary production is limited by light because of the presence of coloured humic substances (cf. Salonen et al. 1983; Jones and Salonen 1985; Kairesalo et al. 1992; del Giorgio and Peters 1993, 1994). On the other hand, the fact that respiration values are high relative to bacterial and primary production rates implies that microorganisms other than bacteria may be largely responsible for community respiration (cf. Kivi et al. 1993).

Table 6. Carbon budget variables for the lake and the enclosures during the autumn experiment (see Table 4 for further details).

	L	C	H	P	HP
Phytoplankton					
Production	12	13	13	21	16
Exudates	3	3	2	3	4
Biomass	42	48	56	62	51
Loss rate	11	10	11	15	10
P:B	0.287	0.271	0.232	0.338	0.314
Bacteria					
Production	3	5	9	7	8
Biomass	22	20	30	25	25
Loss rate	5	5	8	6	7
P:B	0.136	0.250	0.300	0.280	0.320
Protozoa					
Production	3	4	4	4	4
% HNF	30	18	28	25	22
% Ciliata	70	82	72	75	78
Biomass	8	9	12	8	8
P:B	0.375	0.444	0.333	0.500	0.500
Metazooplankton					
Production	0.2	0.4	0.5	0.3	0.4
% Rotatoria	29	37	36	54	39
% Cladocera	5	2	2	2	1
% Copepoda	66	61	62	44	60
Biomass	8	7	12	6	9
P:B	0.025	0.057	0.042	0.050	0.044
Community respiration	36	26	36	44	34
Sedimentation	25	15	29	16	24

In spring and summer, the grazing on bacteria was equal to bacterial production or slightly exceeded it in all treatments, and hence no significant differences in bacterial biomasses could be found. In autumn, the loss rates of bacteria equalled the production rates, but other factors besides grazing (such as cell lysis and sedimentation) probably also affected the loss rates. This conclusion is supported by Servais et al. (1989), who noticed that during the most productive periods (i.e., in spring and in summer) grazing explains the majority of the bacterial mortality, whereas when production is low mortality from grazing is of less importance. The extent to which viral lysis affects the mortality of bacteria in aquatic ecosystems is still largely unknown (Klut and Stockner 1990; Bratbak et al. 1992).

Protozoans, especially HNF, were the most important grazers of bacteria, whereas the direct consumption of bacteria by metazooplankton was insignificant. This is consistent with the hypotheses of Stockner and Porter (1988) and Pace et al. (1990), who stated that the structure of the planktonic community determines the fate of bacterial production: when large *Daphnia* spp. dominate, a considerable proportion of the carbon bound in bacteria is transferred directly to higher trophic levels (e.g., Pedros-Alio and Brock 1983; Güde 1988; Kankaala 1988; Salonen et al. 1992*b*) whereas without large *Daphnia* spp. more energy is dissipated through respiration as

© 1996 NRC Canada

Fig. 3. Flow of carbon in the pelagic community in the spring experiment in control, P, and HP enclosures. Biomasses (*B*) are given as milligrams of carbon per cubic metre, and production (*P*), respiration, and sedimentation rates as milligrams of carbon per cubic metre per day.

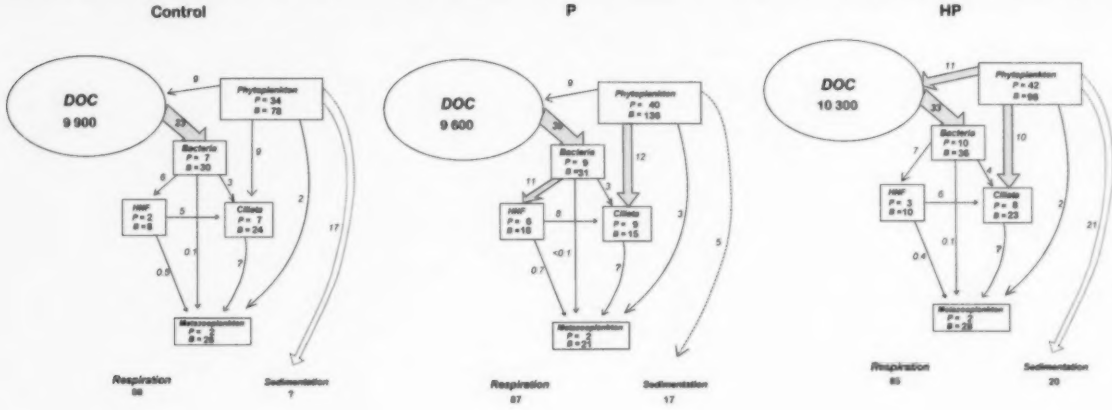


Fig. 4. Flow of carbon in the pelagic community in the summer experiment. See caption of Fig. 3 for details.

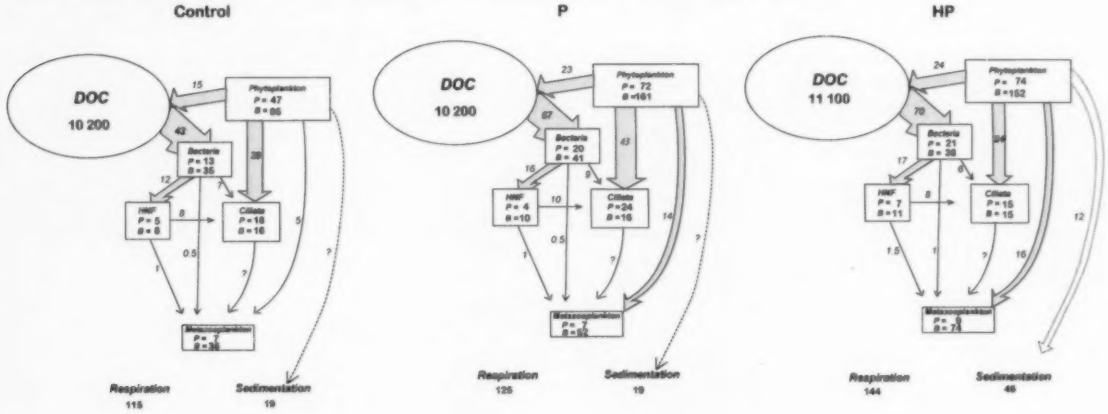
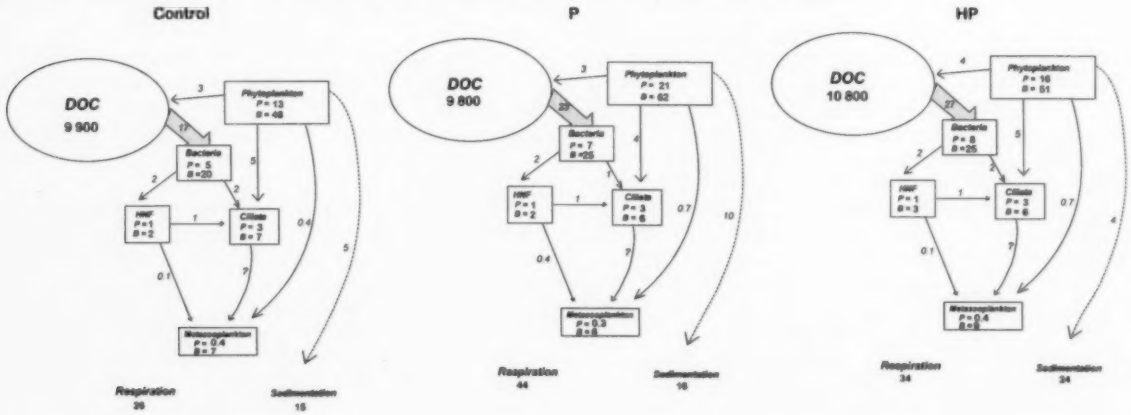


Fig. 5. Flow of carbon in the pelagic community in the autumn experiment. See caption of Fig. 3 for details.



© 1996 NRC Canada

energy is transferred via several steps of the microbial food web (bacteria to HNF to ciliates to metazooplankton) (Sanders et al. 1989; Pace et al. 1990).

The grazing impact of ciliates on algal primary production (18–50% in spring, 20–60% in summer, and 19–38% in autumn) is comparable to the highest estimates for microzooplankton in marine ecosystems (see Pierce and Turner 1992 for a review). Although the importance of ciliates to total zooplankton biomass and production has been demonstrated in some lakes (Pace and Orcutt 1981; Carrick and Fahnenstiel 1990; Geller et al. 1991; Taylor and Johansson 1991), they are rarely included in carbon budgets. Weisse et al. (1990) estimated that ciliates consumed 14% of the spring phytoplankton bloom in Lake Constance. Sherr et al. (1991) found picoplankton-ingesting ciliates in abundance in autumn communities of plankton in Lake Kinneret, and they estimated that the daily requirements of ciliates for growth equalled total primary production. We also estimated ciliate production by multiplying the daily ciliate biomass values by the k_{max} values calculated from the equations of Montagnes et al. (1988) and Müller and Geller (1993), which use ciliate volumes and temperature as independent variables. These production values were 76–91% of the grazing values of ciliates estimated with the model of Peters (1994) applied in this study. Although such high gross growth efficiencies (up to 76–95%) for ciliates have been reported (see Laybourn-Parry 1992 for a review), it is unrealistic to believe that the conditions prevailing in our experiments would have been optimal all the time. As k_{max} values from the equation of Montagnes et al. (1988) based on data of Fenchel (1968) and Finlay (1978) as well as k_{max} values of Müller and Geller (1993) were determined under optimal food conditions, the equations probably overestimate ciliate production in our experiments. Prey density and cell size are taken into consideration in the grazing model of Peters (1994), which thus gives a more realistic estimate of grazing and production (40% gross growth efficiency assumed according to Fenchel 1987). However, the latter estimates can also be too high, as the food selection of ciliates was assumed to depend only on the size and form of food particles (see Methods), and not at all on the chemical properities of the particles (see Pierce and Turner 1992 for a review). It is also possible that the largest ciliates (equivalent spherical diameter > 50 μm) may select food particles of a narrower size spectrum than the smaller ones (Peters 1994). We assumed that algae less than 10^3 μm^3 (equivalent spherical diameter about 12 μm) were grazed by ciliates, although we do not know the proportion of primary production in different size-classes.

The measured clearance rates by metazooplankton in our experiments were low (maximum 17–18% of the water volume per day in summer) compared with many studies, in which maximum rates are >100%/day (e.g., Haney 1973; Knoechel and Holtby 1986*a*). High rates may be due to the use of a single, highly edible food label in grazing experiments (Lampert 1988; Cyr and Pace 1992). For comparison, clearance rates of cladocerans were estimated from the clearance rate – length regression equation of Knoechel and Holtby (1986*b*), and the clearance rates for rotifers and the dominant copepod, *Eudiaptomus gracilis*, were estimated from the average daily clearance rates of Bogdan and Gilbert (1982) and Zankai and Ponyi (1986), respectively. This gave maximum clearance rates of 7–10%/day. Thus, we are confident that the clearance rates by metazooplankton obtained in our study are of the right order of magnitude.

The ingestion rates of metazooplankton in our study may be underestimates because we assumed that algae and HNF <25 μm were cleared with the same efficiency as the labelled *Chlamydomonas* sp. Most rotifers and copepods, as well as bosminid cladocerans, which dominated the metazooplankton community in Lake Pääjärvi (Arvola et al. 1996), are selective feeders that prefer cryptophytes in their food (e.g., Bogdan and Gilbert 1982; Kinsely and Geller 1986). About half of the phytoplankton biomass in spring and summer experiments consisted of cryptophytes and they may have been preferred to other algae. The ingestion of HNF may also be underestimated, as Carrick et al. (1991) found that the clearance rates of macrozooplankton on HNF in Lake Michigan were almost twice the clearance rates on phytoplankton. The importance of ciliates in the food of metazooplankton was not estimated in our experiments, although ciliates have been shown to be intensively grazed by metazooplankton (e.g., Sanders and Wickham 1993) and the clearance rate by suspension-feeding zooplankton on ciliates may be higher than on phytoplankton (Stoecker and Capuzzo 1990). In our experiments there was no correlation between biomasses of protozoan and metazoan plankton. However, the mean volume of ciliates was lowest in summer, when metazooplankton biomass was highest (Arvola et al. 1996). In the HP enclosure, which had the highest metazooplankton biomasses at the end of both spring and summer experiments, the daily values of mean ciliate volume (range 650 – 10 000 μm^3) were negatively correlated to metazooplankton biomass ($r^2 = 0.51$, $p < 0.01$, df = 10). This is in agreement with the results of Wiackowski et al. (1994) who showed that the relative frequency of large ciliates (20 000 μm^3) was depressed and the relative frequency of small ciliates (5000 μm^3) increased in the presence of crustacean zooplankton. The high productivity of ciliates and their role as a potential food source for metazooplankton highlights the importance of protozoans in the food webs of humic lakes, a point deserving more detailed study in the future.

Hessen et al. (1989, 1990) argued that particulate detritus is a very important source of food for zooplankton in the Norwegian humic lake Kjelsåsputten. They estimated that particulate detritus formed 75–90% of POC (POC concentration 0.6–0.7 mg C·L^{-1}). In our experiments the POC concentration was lower (on average 0.2 mg C·L^{-1} in control and 0.3 mg C·L^{-1} in HP) and the proportion of detrital POC was <10–30% in spring and summer and 56–57% in autumn. However, the carbon conversion factors applied affect the results and conclusions: if carbon:volume ratios of 10 and 12% were used for algae and bacteria, respectively (Hessen et al. 1989, 1990), the proportion of detrital particles in POC would exceed 50% in spring and summer. Accordingly the algal and bacterial carbon would not be enough to support zooplankton production. The carbon:volume ratio of bacteria used in our experiments was 36% and was based on our own measurements (Tulonen 1993). The carbon:volume ratio of 20% used for algae and protozoans has been widely accepted (Børsheim and Bratbak 1987; Rocha and Duncan 1985; Verity et al. 1992). Hessen et al. (1990) did not estimate the role of protozooplankton in the carbon fluxes of Lake Kjelsåsputten. In steady-state radiotracer experiments, Salonen and Hammar (1986) found that most carbon (about

© 1996 NRC Canada

60% in light and about 95% in dark) in planktonic crustaceans in humic lakes originated from allochthonous DOC. Our results emphasize the important role of protozooplankton in the food of crustaceans, but most of the allochthonous carbon, taken up by bacteria and transferred further via HNF and ciliates, is lost through respiration.

One explanation for the discrepancy between the findings of the present study and the results of Salonen and Hammar (1986) might be that the conditions in the bottle experiments of Salonen and Hammar (1986) may have favoured the growth of rapidly growing protozoan flagellates (cf. Goldman and Caron 1985; Carrick et al. 1992). Their results probably indicate the maximum proportion of food originating from allochthonous DOM, but the proportion may be lower in the euphotic zone of humic lakes. It is also possible that growth efficiencies of metazooplankton in humic lakes are higher than in clear-water lakes, as cryptophytes, known to be high-quality food for zooplankton (Stemberger 1981; Skogstad et al. 1987; Ahlgren et al. 1990; Lundstedt and Brett 1991), are often abundant in this type of lake (cf. Eloranta 1986; Ilmavirta 1990).

In calculations of carbon budget based on studies in the early 1970s, Sarvala et al. (1981) estimated that phytoplankton production in Lake Pääjärvi is not sufficient to support the food requirements of herbivorous zooplankton, and that bacteria must therefore serve as an additional food source. The results of those studies are not necessarily inconsistent with our results, as the role of the microbial food chain was unknown in the 1970s. Our experiments were carried out in the uppermost 3 m of the water column, where ca. 95% of primary production takes place (T. Tulonen, unpublished data). The zooplankton is, however, distributed throughout the whole water column. According to the results of Latja (R.L. Eteläkatu, 16 A 4, FIN–80110 Joensuu, Finland, unpublished data) about half of the metazooplankton biomass appeared in the epilimnion (0–15 m), but only about 20% was found in uppermost 3-m layer in the 60 m deep central part of the lake. Because our enclosures were filled in daylight and samples in the lake were always taken during the day, some of the vertically migrating zooplankton were probably excluded in our study. According to T. Tulonen (unpublished data), the biomass of protozoans below the euphotic zone (10–50 m) is of the same order of magnitude (7–20 mg $C \cdot m^{-3}$) as in the euphotic zone (5–31 mg $C \cdot m^{-3}$, see also Tables 4–5). Thus, the channeling of allochthonous carbon via bacteria and protozans to metazooplankton may be very important below the euphotic zone.

The carbon budgets are sensitive to conversion factors, and more detailed studies on the growth efficiencies of the organisms at different trophic levels utilizing various food sources (both autochthonous and allochthonous) are needed. Provided that the compartments of the carbon budgets of this study are in correct proportion to each other, we can conclude that the expected climate change in the boreal zone will influence more strongly the microbial community than higher trophic levels. Thus, increased leaching of nutrients and DOM from the catchments into lakes will result in increased community respiration, and thus the imbalance between respiration and fixation of carbon can be expected to increase.

Acknowledgements

This study is part of the Finnish Research Programme on Climate Change (SILMU) and was funded by the Academy of Finland. We thank R.I. Jones for comments and correcting the language of the manuscript. The constructive criticism of two referees also improved this paper.

References

Ahlgren, G., Lundstedt, L., Brett, M., and Forsberg, C. 1990. Lipid composition of some freshwater phytoplankton for cladoceran zooplankters. J. Plankton Res. **12**: 809–818.

Arvola, L., Salonen, K., and Rask, M. 1990. Chemical budgets for a small dystrophic lake in southern Finland. Limnologica, **20**: 243–251.

Arvola, L., Kankaala, P., Tulonen, T., and Ojala, A. 1996. Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake (Lake Pääjärvi). Can. J. Fish. Aquat. Sci. **53**: 1646–1662.

Benner, R., Lay, J., K'nees, E., and Hodson, R.E. 1988. Carbon conversion efficiency for bacterial growth on lignocellulose: implication for detritus-based food webs. Limnol. Oceanogr. **33**: 1514–1526.

Boer, M.M., Koster, E.A., and Lundberg, H. 1990. Greenhouse impact in Fennoscandia: preliminary findings of a European workshop on the effects of cilimatic change. Ambio, **19**: 2–10.

Bogdan, K.G., and Gilbert, J.J. 1982. Seasonal patterns of feeding by natural populations of *Keratella*, *Polyarthra*, and *Bosmina*: clearance rates, selectivities, and contributions to community grazing. Limnol. Oceanogr. **27**: 918–934.

Børsheim, K.Y., and Bratbak, G. 1987. Cell volume to cell carbon conversion factors for a bacteriovorous *Monas* sp. enriched from seawater. Mar. Ecol. Prog. Ser. **36**: 171–175.

Bottrell, H.H., Duncan, A., Gliwicz, Z.M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurasawa, H., Larsson, P., and Weglenska, T. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. **24**: 419–456.

Bratbak, G., Heldal, M., Thingstad, T.F., Riemann, B., and Haslund, O.H. 1992. Incorporation of viruses into the budget of microbial C-transfer. A first approach. Mar. Ecol. Prog. Ser. **83**: 273–280.

Carrick, H.J., and Fahnenstiel, G.L. 1990. Planktonic protozoa in lakes Huron and Michigan: seasonal abundance and composition of ciliates and dinoflagellates. J. Great Lakes Res. **16**: 319–329.

Carrick, H.J., Fahnenstiel, G.L., Stoermer, E.F., and Wetzel, R.G. 1991. The importance of zooplankton-protozoan couplings in Lake Michigan. Limnol. Oceanogr. **36**: 1335–1345.

Carrick, H.J., Fahnenstiel, G.L., and Taylor, W.D. 1992. Growth and production of planktonic protozoa in Lake Michigan: in situ versus in vitro comparisons and importance to food web dynamics. Limnol. Oceanogr. **37**: 1221–1235.

Coffin, R.B., Conolly, J.P., and Harris, P.S. 1993. Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. Mar. Ecol. Prog. Ser. **101**: 9–22.

Cole, J.J., Findlay, S., and Pace, M.L. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. **43**: 1–10.

Cyr, H., and Pace, M.L. 1992. Grazing by zooplankton and its relationship to community structure. Can. J. Fish. Aquat. Sci. **49**: 1455–1465.

Del Giorgio, P.A., and Peters, R.H. 1993. Balance between phytoplankton production and plankton respiration rates in lakes. Can. J. Fish. Aquat. Sci. **50**: 282–289.

Del Giorgio, P.A., and Peters, R.H. 1994. Patterns in planktonic P:R ratios in lakes: influence of lake trophy and dissolved organic carbon. Limnol. Oceanogr. **39**: 772–787.

© 1996 NRC Canada

Eloranta, P. 1986. Phytoplankton structure in different lake types in central Finland. Holarct. Ecol. **9**: 214–224.

Fenchel, T. 1968. The ecology of marine microbenthos. III. The reproductive potential of ciliates. Ophelia, **5**: 123–126.

Fenchel, T. 1987. Ecology of protozoa. The biology of free-living phagotrophic protists. Springer-Verlag. Madison, Wis.

Findlay, S., Carlough, L., Crocker, M.T., Gill, H.K., Meyer, J.L., and Smith, P.J. 1986. Bacterial growth on macrophyte leachate and fate of bacterial production. Limnol. Oceanogr. **31**: 1335–1341.

Finlay, B.J. 1978. Community production and respiration by ciliated protozoa in the benthos of a small eutrophic loch. Freshwater Biol. **8**: 327–341.

Garnier, J., and Mourelatos, S. 1991. Contribution of grazing in phytoplankton overall losses in a shallow French lake. Freshwater Biol. **25**: 515–523.

Geller, W., Berberovic, R., Gaedke, U., Müller, H., Pauli, H.-R., Tilzer, M.M., and Weisse, T. 1991. Relations among the components of autotrophic and heterotrophic plankton during the seasonal cycle 1987 in Lake Constance. Verh. Int. Ver. Theor. Angew. Limnol. **24**: 831–836.

Goldman, J.C., and Caron, D.A. 1985. Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. Deep-Sea Res. **32**: 899–915.

Güde, H. 1988. Direct and indirect influences of crustacean zooplankton on bacterioplankton on Lake Constance. Hydrobiologia, **159**: 63–73.

Haney, J.F. 1973. An in situ examination of the grazing activities of natural zooplankton communities. Arch. Hydrobiol. **72**: 87–132.

Hessen, D., Andersen, T., and Lyche, A. 1989. Differential grazing and resource utilization of zooplankton in a humic lake. Arch. Hydrobiol. **114**: 321–347.

Hessen, D., Andersen, T., and Lyche, A. 1990. Carbon metabolism in a humic lake: pool sizes and cycling throgh zooplankton. Limnol. Oceanogr. **35**: 84–99.

Holtby, L.B., and Knoechel, R. 1981. Zooplankton filtering rates: error due to loss of radioisotopic label in chemically preserved samples. Limnol. Oceanogr. **26**: 774–780.

Ilmavirta, V. 1990. Occurrence of phytoplankton species along nutrient, pH and colour gradients in Eastern Finnish lakes. Verh. Int. Ver. Theor. Angew. Limnol. **24**: 702–706.

Intergovernmental panel on climate change. 1990. Climate change: the IPCC scientific assessment. Cambridge University Press, Cambridge, U.K.

Jones, R.I., and Salonen, K. 1985. The importance of bacterial utilization of released phytoplankton photosynthate in two humic forest lakes in southern Finland. Holarct. Ecol. **8**: 133–140.

Kairesalo,T., Lehtovaara, A., and Saukkonen, P. 1992. Littoral–pelagial interchange and the decomposition of dissolved organic matter in a polyhumic lake. Hydrobiologia, **229**: 199–224.

Kankaala, P. 1988. The relative importance of algae and bacteria as food for *Daphnia longispina* (Cladocera) in a polyhumic lake. Freshwater Biol. **19**: 285–296.

Kankaala, P., and Wulff, F. 1981. Experimental studies on temperature-dependent embryonic and postembryonic developmental rates of *Bosmina longispina maritima* (Cladocera) in the Baltic. Oikos, **36**: 137–146.

Kinsely, K., and Geller, W. 1986. Selective feeding of four zooplankton species on natural lake phytoplankton. Oecologia, **69**: 86–94.

Kivi, K., Kaitala, S., Kuosa, H., Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, B., and Tamminen, T. 1993. Nutrient limitation and grazing control of the Baltic plankton community during annual succession. Limnol. Oceanogr. **38**: 893–905.

Klut, M.E., and Stockner, J.B. 1990. Virus-like particles in an ultra-oligotrophic lake on Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. **47**: 725–730.

Knoechel R., and Holtby, L.B. 1986*a*. Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. Limnol. Oceanogr. **31**: 1–16.

Knoechel, R., and Holtby, L.B. 1986*b*. Cladoceran filtering rate: body length relationships for bacterial and large algal particles. Limnol. Oceanogr. **31**: 195–200.

Kristiansen, K., Nielsen, H., Riemann, B., and Fuhrman, J.A. 1992. Growth efficiencies of freshwater bacterioplankton. Microb. Ecol. **24**: 145–160.

Kroer, N. 1993. Bacterial growth efficiency on natural dissolved organic matter. Limnol. Oceanogr. **38**: 1282–1290

Lampert, W. 1988. The relationship between zooplankton biomass and grazing: a review. Limnologica, **19**: 11–20.

Larsson, U., and Hagström, Å. 1982. Fractionated phytoplankton primary production, exudate release, and bacterial production in a Baltic eutrophication gradient. Mar. Biol. (Berlin), **67**: 57–70.

Laybourn-Parry, J. 1992. Protozoan plankton ecology. Chapman and Hall, London.

Lignell, R., Heiskanen, A.-S., Kuosa, H., Gundersen, K., Kuuppo-Leinikki, P., Pajuniemi, R., and Uitto, A. 1993. Fate of a phytoplankton spring bloom: sedimentation and carbon flow in the planktonic food web in the northern Baltic. Mar. Ecol. Prog. Ser. **94**: 239–252.

Makarewicz, J.C., and Likens, G.E. 1979. Structure and function of zooplankton community of Mirror Lake, New Hampshire. Ecol. Monogr. **49**: 109–127.

Meili, M., Fry, B., and Kling, G.W. 1993. Fractionation of stable isotopes (^{13}C, ^{15}N) in the food web of a humic lake. Verh. Int. Ver. Theor. Angew. Limnol. **25**: 501–505.

Meyer, J., Edwards, R.T., and Risley, R. 1987. Bacterial growth on dissolved organic carbon from a blackwater river. Microb. Ecol. **13**: 13–29.

Montagnes, D.J.S., Lynn, D.H., Roff, J.C., and Taylor, W.D. 1988. The annual cycle of heterotrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assesesment of their trophic role. Mar. Biol. (Berlin), **99**: 21–31.

Müller, H., and Geller, W. 1993. Maximum growth rates of aquatic ciliated protozoa: the dependence on body size and temperature reconsidered. Arch. Hydrobiol. **126**: 315–327.

O'Brien, W.J., Hershey, A.E., Hobbie, J.E., Hullar, M.A., Kipphut, G.W., Miller, M.C., Moller, B., and Vestal, J.R. 1992. Control mechanisms of arctic lake ecosystems: a limnocorral experiment. Hydrobiologia, **240**: 143–188.

Pace, M.L., and Orcutt, J.D., Jr. 1981. The relative importance of protozoans, rotifers, and crrustaceans in a freshwater zooplankton community. Limnol Oceanogr. **26**: 822–830.

Pace, M.L., McManus, G.B., and Findlay, S.E.G. 1990. Planktonic community structure determines the fate of bacterial production in a temperate lake. Limnol. Oceanogr. **35**: 795–808.

Pedros-Alio, M.L., and Brock, T.D. 1983. The impact of zooplankton feeding on the epilimnetic bacteria of an eutrophic lake. Freshwater Biol. **13**: 227–239.

Peters, F. 1994. Prediction of planktonic protistan grazing rates. Limnol. Oceanogr. **39**: 195–206.

Pierce, R.W., and Turner, J.T. 1992. Ecology of planktonic ciliates in marine food webs. Rev. Aquat. Sci. **6**: 139–181.

Pomeroy, L.R., and Wiebe, W.J. 1988. Energetics of microbial food webs. Hydrobiologia, **159**: 7–18.

Rocha, O., and Duncan, A. 1985. Relationship between cell carbon and cell volume in freshwater algae species used in zooplankton studies. J. Plankton Res. **7**: 279–294.

Salonen, K., and Hammar, T. 1986. On the importance of dissolved organic matter in the nutrition of zooplankton in some lake waters. Oecologia, **68**: 246–253.

Salonen, K., Kononen, K., and Arvola, L. 1983. Respiration of plankton in two small, polyhumic lakes. Hydrobiologia, **101**: 65–70.

Salonen, K., Arvola, L., Tulonen, T., Hammar, T., Metsälä, T.-R., Kankaala, P., and Münster, U. 1992*a*. Planktonic food chains in a

© 1996 NRC Canada

highly humic lake. I. A mesocosm experiment during the spring primary production maximum. Hydrobiologia, **229**: 125–142.

Salonen, K., Kankaala, P., Tulonen, T., Hammar, T., James, M., Metsälä, T.-R., and Arvola, L. 1992*b*. Planktonic food chains in a highly humic lakes. II. A mesocosm experiment in summer during dominance of heterotrophic processes. Hydrobiologia, **229**: 143–157.

Sanders, R.W., and Wickham, S.A. 1993. Planktonic protozoa and metazoa: predation, food quality and population control. Mar. Microb. Food Webs, **7**: 197–223.

Sanders, R.W., Porter, K.G., Bennett, S.J., and DeBiase, A.E. 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers and cladocerans in a freshwater plankton community. Limnol. Oceanogr. **34**: 673–687.

Sarvala, J., Ilmavirta, V., Paasivirta, L., and Salonen, K. 1981. The ecosystem of the oligotrophic Lake Pääjärvi. 3. Secondary production and an ecological energy budget of the lake. Verh. Int. Ver. Theor. Angew. Limnol. **21**: 422–427.

Servais, P., Billen, G., Martinez, J., and Vives-Rego, J. 1989. Estimating bacterial mortality by the disappearance of ^{3}H-labeled intracellular DNA. FEMS Microbiol. Ecol. **62**: 119–126.

Sherr, E.B., Sherr, B.F., Berman, T., and Hadas, O. 1991. High abundance of picoplankton-ingesting ciliates during late fall in Lake Kinneret, Israel. J. Plankton Res. **13**: 789–799.

Simon, M., and Tilzer, M.M. 1987. Bacterial response to seasonal changes in primary production and phytoplankton biomass in Lake Constance. J. Plankton Res. **9**: 535–553.

Skogstad, A., Granskog, L., and Klaveness, D. 1987. Growth of freshwater ciliates offered planktonic algae as food. J. Plankton Res. **9**: 503–512.

Stemberger, R.S. 1981. A general approach to the culture of planktonic rotifers. Can. J. Fish. Aquat. Sci. **38**: 721–724.

Stockner, J.G., and Porter, K.G. 1988. Microbial food webs in freshwater planktonic ecosystems. *In* Complex interactions in lake communities. *Edited by* S.R. Carpenter. Springer-Verlag, New York. pp. 69–83.

Stoecker, D.K., and Capuzzo, J.M. 1990. Predation on protozoa: its importance to zooplankton. J. Plankton Res. **12**: 891–908.

Sundh, I., and Bell, R.T. 1992. Extracellular dissolved organic carbon released from phytoplankton as a source of carbon for heterotrophic bacteria in lakes of different humic content. Hydrobiologia, **229**: 93–106.

Søndergaard, M., Riemann, B., Jensen, L.M., Jørgensen, N.O.G., Bjørnsen, P.K., Olesen, M., Larsen, J.B., Geertz-Hensen, O., Hansen, J., Christoffersen, K., Jespersen, A.-M., Andersen, F., and Bosselman, S. 1988. Pelagic food web processes in an oligotrophic lake. Hydrobiologia, **164**: 271–286.

Taylor, W.D., and Johannsson, O.E. 1991. A comparison of estimates of productivity and consumption by zooplankton for planktonic ciliates in Lake Ontario. J. Plankton Res. **13**: 363–372.

Thompson, A.S., Rhodes, J.C., and Pettman, I. 1988. Culture collection of algae and protozoa. Catalogue of strains. 5th ed. Natural Environmental Research Council, CCAP, Ambleside, U.K.

Tranvik, L.J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb. Ecol. **16**: 311–322.

Tranvik, L.J. 1990. Bacterioplankton growth on fractions of dissolved organic carbon of different molecular weights from humic and clear waters. Appl. Environ. Microbiol. **56**: 1672–1677.

Tranvik, L. 1994. Effects of colloidal organic matter on the growth of bacteria and protists in lake water. Limnol. Oceanogr. **39**: 1276–1285.

Tranvik, L.J., and Höfle, M.G. 1987. Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. Appl. Environ. Microbiol. **53**: 482–488.

Tulonen, T. 1993. Bacterial production in a mesohumic lake estimated from (^{14}C)-leucine incorporation rate. Microb. Ecol. **26**: 201–217.

Tulonen, T., Salonen, K., and Arvola, L. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. Hydrobiologia, **229**: 239–252.

Vadstein, O., Harkjerr, B.O., Jensen, A., Olsen, Y., and Reinertsen, H. 1989. Cycling of organic carbon in the photic zone of a eutrophic lake with special reference to the heterotrophic bacteria. Limnol. Oceanogr. **34**: 840–855.

Verity, P.G., Robertson, C.Y., Tronzo, C.R., Andrews, M.G., Nelson, J.R., and Sieracki, M.E. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. **37**: 1434–1446.

Vijverberg, J. 1980. Effect of temperature in laboratory studies of development and growth of Cladocera and Copepoda from Tjeukemeer, the Netherlands. Freshwater Biol. **10**: 317–340.

Weisse, T., Müller, H., Pinto-Coelho, M., Schweizer, A., Springmann, D., and Baldringer, G. 1990. Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnol. Oceanogr. **35**: 781–794.

Wiackowski, K., Brett, M.T., and Goldman, C.R. 1994. Differential effects of zooplankton species on ciliate community structure. Limnol. Oceanogr. **39**: 486–492.

Winberg, G.G. 1971. Methods for the estimation of production of aquatic animals. Academic Press, London.

Zánkai, P.N., and Ponyi, J.E. 1986. Composition, density and feeding of crustacean zooplankton community in a shallow, temperate lake (Lake Balaton, Hungary). Hydrobiologia, **135**: 131–147.

© 1996 NRC Canada

How does somatic growth rate affect otolith size in fishes?

Yongshun Xiao

Abstract: Although much sought, relationships between fish otolith size and somatic growth rate have not been established. This paper derives otolith size as a function of somatic growth rate from allometric relations of otolith size to body size and from the von Bertalanffy, logistic, and Gompertz (somatic) growth equations for an individual fish: generally, these relationships can be given analytically and (or) numerically. The relationships predict that for constant allometric parameters, otolith size decreases steadily with somatic growth rate for an individual growing according to the von Bertalanffy equation, whereas it first increases and then decreases with somatic growth rate for an individual growing according to the logistic or Gompertz equation. They are then used to reveal the complex relationships between fish otolith size and somatic growth rate in a case study for age-varying allometric parameters and in a simulation study for a group of individuals with different growth parameters. Because relationships between otolith size and somatic growth rate can be evaluated analytically and (or) numerically, this study provides an alternative approach to experimental study of otolith size as a function of somatic growth rate.

Résumé : Malgré toutes les recherches menées sur le sujet, on n'a pas encore établi de relations directes entre la taille des otolithes de poissons et le taux de croissance somatique. Nous calculons ici la taille de otolithes comme fonction du taux de croissance somatique à partir des relations allométriques entre la taille de l'otolithe et la taille du corps, et à partir d'équations de la croissance (somatique) de von Bertalanffy, logistique et de Gompertz pour les poissons pris individuellement : de façon générale, ces relations peuvent être données sous forme analytique et (ou) numérique. Les relations prédisent que, pour des paramètres allométriques constants, la taille de l'otolithe diminue régulièrement en fonction du taux de croissance somatique pour un individu en croissance selon l'équation de von Bertalanffy, tandis qu'elle augmente d'abord pour diminuer ensuite en fonction du taux de croissance somatique pour un individu en croissance selon une courbe logistique ou une courbe de Gompertz. On se sert ensuite de ces résultats pour faire ressortir les relations complexes entre la taille de l'otolithe et le taux de croissance somatique dans une étude de cas dont les paramètres allométriques varient en fonction de l'âge, et dans une étude de simulation pour un groupe d'individus dont les paramètres de croissance sont divers. Étant donné que les relations entre la taille de l'otolithe et le taux de croissance somatique peuvent être évalués de façon analytique et (ou) numérique, notre recherche présente une autre possibilité d'étudier expérimentalement la taille de l'otolithe en fonction du taux de croissance somatique.
[Traduit par la Rédaction]

Introduction

Relationships between otolith size and somatic growth rate of fishes are commonly considered useful for correcting biases in back-calculated lengths at age and for indexing somatic growth rate (McGurk 1984; Reznick et al. 1989; Secor and Dean 1989, 1992; Campana 1990); they have been the subject of much experimentation (Templeman and Squires 1956; Marshall and Parker 1982; Mosegaard et al. 1988; Reznick et al. 1989; Secor and Dean 1989, 1992; Secor et al. 1989; Campana 1990; Molony and Choat 1990; Wright et al. 1990; Francis et al. 1993). Qualitative evidence that somatic growth rate affects otolith size in different individuals was obtained by analysis of variance or covariance in larval sockeye salmon (*Oncorhynchus nerka*; Marshall and Parker 1982), Arctic char (*Salvelinus alpinus*; Mosegaard et al. 1988), guppies (*Poecilia reticulata*; Reznick et al. 1989), larval and juvenile striped bass (*Morone saxatilis*; Secor and Dean 1989, 1992), glass fish (*Ambassis vachelli*; Molony and Choat 1990), larval and juvenile red sea-bream (*Pagrus major*; Secor et al. 1989), juvenile spot (*Leiostomus xanthurus*; Secor et al. 1989), and juvenile "snapper" (*Pagrus auratus*; Francis et al. 1993). Quantitative evidence was provided by the demonstration of an empirical relationship between the ratio of otolith size to somatic size and somatic growth rate in larval and juvenile striped bass (*Morone saxatilis*; Secor et al. 1989). Under certain assumptions about somatic growth, this empirical relationship applies for its intended fish over the range of somatic growth rate studied (<1.5 mm·d^{-1}), but it cannot be generally extrapolated to other stages or species. Those studies have led to an inference that slow-growing fish individuals generally have larger otoliths than fast-growing individuals of the same length. Thus, direct, general relationships between the size of otoliths, scales, and other hard parts and somatic growth rate have not yet been established for different individuals of any fish species. Because of experimental constraints on the somatic growth rates that can be studied, one must consider whether such general relationships can be deduced from our knowledge of fish growth. If these relationships can be derived, they could

Received January 25, 1994. Accepted January 19, 1996.
J12250

Y. Xiao.[1] Fisheries Division, Department of Primary Industry and Fisheries, GPO Box 990, Darwin, Northern Territory 0801, Australia.

[1] Present address: CSIRO Division of Fisheries, GPO Box 1538, Hobart, Tasmania 7001, Australia. e-mail: xiao@ml.csiro.au

© 1996 NRC Canada

generate predictions over the entire range of somatic growth rate, they might lead to critical reevaluations of previous work, and they might be useful for correcting biases in back-calculated lengths at age and for indexing somatic growth rate by using otolith size.

In this paper, I will show that direct, general relationships between somatic growth rate and the size of a hard part of a single individual (or a group of individuals with an identical set of somatic and otolith growth parameters) can be deduced from known somatic growth equations and equations relating the size of that hard part to somatic size. As an example, I will derive fish otolith size as a function of somatic growth rate from allometric relations of otolith to body size and from the von Bertalanffy, logistic, and Gompertz growth equations. The derived relationships link otolith size to somatic growth rate through somatic growth parameters and parameters in otolith size and somatic size relations. They predict that for constant allometric parameters, the otolith size of an individual growing according to the von Bertalanffy equation decreases with its somatic growth rate, whereas that of an individual growing according to the logistic or Gompertz equation first increases and then decreases with it. These relationships are then used to reveal the complex relationships between fish otolith size and somatic growth rate in a case study for age-varying allometric parameters and in a simulation study of otolith size as a function of somatic growth rate for a group of individuals with different somatic growth parameters. Because relationships between otolith size and somatic growth rate can be evaluated, this study provides an alternative approach to experimental study of otolith size as a function of somatic growth rate. For convenience, I will consider fish otoliths only, although the method can also be applied to other hard parts of fishes and other animals.

Model

Let $L = L(t)$ be a general somatic growth function of age of a single fish (or a group of fish with an identical set of growth parameters) t (e.g., von Bertalanffy, logistic, and Gompertz equations), such that its otolith size $S = S(t,L)$ and $R = S/L$. The somatic growth rate of that animal (or group) is then given by the first derivative of $L(t)$ with respect to t, i.e., $\dot{L}(t)$. Note that growth rates estimated as difference quotients (increments or decrements over a certain interval of age) are only approximations because of the way the first derivative is defined. The problem of finding a relationship between otolith size and somatic growth rate then becomes to solve

$$(1.0)\quad \begin{cases} S = S(t,L) \\ L = L(t) \end{cases}$$

for $S = S(\dot{L})$. An analytical solution is certainly possible for some forms of $S(t,L)$ and $L(t)$, while a numerical solution is necessary for others. For those forms with explicit analytical solutions, this problem may be approached by (*i*) choosing appropriate forms of $L(t)$ usually in differential equations and $S(t,L)$ usually as a direct function of t and L; (*ii*) solving for $L = L(t)$, $t = t(L)$, and $L = L(\dot{L})$; (*iii*) successively substituting $t = t(L)$ and $L = L(\dot{L})$ into $S(t,L)$ for $S = S(\dot{L})$; and finally (*iv*) evaluating $S = S(\dot{L})$ in terms of $\dot{L}$. If $L = L(t)$, $t = t(L)$, or $L = L(\dot{L})$ is not solvable explicitly analytically, $S = S(\dot{L})$ can be evaluated by calculus or numerically. I now demonstrate this approach by developing relationships between fish otolith size and somatic growth rate, with and without explicit analytical solutions. It must be noted that relationships between fish somatic growth rate and scale size may be more complex, because scales can not only increase with somatic size but also decrease with it by way of resorption and erosion (Casselman 1990).

Functional forms of $S(t,L)$

Although $S(t,L)$ may take any form such as polynomials of appropriate orders, many biological variables (e.g., shape and size variables) and processes are scaled allometrically to body size. In this illustration, I will use the general allometric equation

$$(2.0)\quad S = c + aL^b$$

(Huxley 1972) to represent relationships between fish otolith size, S, and body size, L, with allometric parameters $a > 0$, $b > 0$, and $c \leq 0$ as functions of fish age t; any other relationships could be examined similarly. A special case of eq. 2.0 for $b = 1$ is a linear curve for a given age t, which is often used to model relationships between otolith and body sizes. In most fishes, sagittal otoliths appear at about the time of hatching, when fish larvae already have a body size of several millimetres ($c \leq 0$) (Campana and Neilson 1985; Campana 1990). If both body and otolith start to grow at precisely the same instant (i.e., $c = 0$), the general allometric equation is reduced to the common allometric equation (Peters 1983; Calder 1984; Schmidt-Nielsen 1984; Reiss 1989). Parameter a is also called the fractional coefficient (Huxley 1972) and satisfies $a > 0$, since biological characteristics generally take nonnegative values. Because otolith size generally increases with somatic size in a single individual (or a group of fish with an identical set of allometric parameters), $b > 0$.

Functional forms of $L(t)$

Many growth models (e.g., von Bertalanffy, logistic, and Gompertz) are adequate for this purpose, including such general and flexible growth models as Sainsbury's (1980) and Schnute's (1981). For illustration, I will choose the most commonly used von Bertalanffy, logistic, and Gompertz growth equations, although a linear or exponential function of age can provide a better description of somatic growth of larval and juvenile fishes. In differential form, the von Bertalanffy, logistic, and Gompertz equations are given by

$$(3.0)\quad \dot{L} = K(L_\infty - L) \quad 0 \leq K, 0 \leq L \leq L_\infty, 0 \leq \dot{L} \leq KL_\infty,$$

$$(3.1)\quad \dot{L} = KL\left(1 - \frac{L}{L_\infty}\right) \quad 0 \leq K, 0 \leq L \leq L_\infty, 0 \leq \dot{L} \leq \frac{1}{4}KL_\infty,$$

and

$$(3.2)\quad \dot{L} = KL\log_e\left(\frac{L_\infty}{L}\right) \quad 0 \leq K, 0 \leq L \leq L_\infty, 0 \leq \dot{L} \leq \frac{1}{e}KL_\infty,$$

respectively, with von Bertalanffy, logistic, or Gompertz parameters (K,L_∞). Note that K and L_∞ have similar meanings in these equations: K is a correlate of animal size and longevity; L_∞ is the asymptotic size of an individual animal. Note also that eqs. 3.0–3.2 apply not only when K and L_∞ are constant but also when they are reasonable functions of fish age t and environmental factors (e.g., water temperature, and food quality

© 1996 NRC Canada

and quantity), such as $K(t) = \alpha + \beta\,(2\,\pi t/T + \phi) \geq 0$, where $\alpha \geq 0$, $\beta \geq 0$, $T > 0$, and ϕ are model parameters.

Functional forms of $L = L(t)$

Solution of eqs. 3.0–3.2 each as an initial value problem with $L(t)\,|_{t=t_0} = L(t_0)$ yields

$$(4.0)\quad L(t) = L(t_0)\,e^{-\int_{t_0}^{t} K(s)ds} + \int_{t_0}^{t} K(s)L_\infty(s)\,e^{-\int_{s}^{t} K(\xi)d\xi}ds,$$

$$(4.1)\quad L(t) = \left(\frac{1}{L(t_0)}\,e^{-\int_{t_0}^{t} K(s)ds} + \int_{t_0}^{t} \frac{K(s)}{L_\infty(s)}\,e^{-\int_{s}^{t} K(\xi)d\xi}ds\right)^{-1},$$

and

$$(4.2)\quad L(t) = L(t_0)^{e^{-\int_{t_0}^{t} K(s)ds}}\, e^{\int_{t_0}^{t} K(s)\log_e(L_\infty(s))\,e^{-\int_{s}^{t} K(\xi)\,d\xi}\,ds},$$

respectively, with $K = K(t)$ and $L_\infty = L_\infty(t)$. If both K and L_∞ are constant, eqs. 3.0–3.2 or eqs. 4.0–4.2 become

$$(5.0)\quad L(t) = L_\infty - (L_\infty - L(t_0))e^{-K(t-t_0)},$$

$$(5.1)\quad L(t) = \frac{L(t_0)L_\infty}{L(t_0) + (L_\infty - L(t_0))e^{-K(t-t_0)}},$$

and

$$(5.2)\quad L(t) = L_\infty\left(\frac{L(t_0)}{L_\infty}\right)^{e^{-K(t-t_0)}},$$

respectively.

Functional forms of $t = t(L)$

Generally, it is impossible to solve eqs. 3.0–3.2 or eqs. 4.0–4.2 in closed form for $t = t(L)$. One can, however, readily solve eqs. 5.0–5.2 for $t = t(L)$ as

$$(6.0)\quad t = t_0 - \frac{1}{K}\log\left(\frac{L_\infty - L(t)}{L_\infty - L(t_0)}\right)$$

for the von Bertalanffy growth equation,

$$(6.1)\quad t = t_0 - \frac{1}{K}\log\left(\frac{L(t_0)(L_\infty - L(t))}{L(t)(L_\infty - L(t_0))}\right)$$

for the logistic growth equation, and

$$(6.2)\quad t = t_0 - \frac{1}{K}\log\left(\frac{\log(L_\infty) - \log(L(t))}{\log(L_\infty) - \log(L(t_0))}\right)$$

for the Gompertz growth equation.

Functional forms of $L = L(\dot{L})$

In the form of $L = L(\dot{L})$, the von Bertalanffy and logistic equations can be written as

$$(7.0)\quad L = L_\infty - \frac{\dot{L}}{K},$$

and

$$(7.1)\quad L = \frac{L_\infty}{2}\left(1 \pm \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right),$$

respectively. For the Gompertz growth equation, direct evaluation by calculus is necessary as $L = L(\dot{L})$ cannot be solved explicitly.

Functional forms of $S = S(\dot{L})$

Substitution of eqs. 6.0 and 6.1 into eq. 2.0 and then of eqs. 7.0 and 7.1 into the resulting equation gives

$$(8.0)\quad S = c\left(t_0 - \frac{1}{K}\log\left(\frac{\dot{L}}{K(L_\infty - L(t_0))}\right)\right) + a\left(t_0 - \frac{1}{K}\log\left(\frac{\dot{L}}{K(L_\infty - L(t_0))}\right)\right)\left(L_\infty - \frac{\dot{L}}{K}\right)^{b\left(t_0 - \frac{1}{K}\log\left(\frac{\dot{L}}{K(L_\infty - L(t_0))}\right)\right)}$$

and

$$(8.1)\quad S = c\left(t_0 - \frac{1}{K}\log\left(\frac{L(t_0)\left(1 \mp \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}{(L_\infty - L(t_0))\left(1 \pm \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}\right)\right) + a\left(t_0 - \frac{1}{K}\log\left(\frac{L(t_0)\left(1 \mp \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}{(L_\infty - L(t_0))\left(1 \pm \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}\right)\right)$$

$$\times\left(\frac{L_\infty}{2}\left(1 \pm \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)\right)^{b\left(t_0 - \frac{1}{K}\log\left(\frac{L(t_0)\left(1 \mp \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}{(L_\infty - L(t_0))\left(1 \pm \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}\right)\right)},$$

respectively. For the Gompertz growth equation, direct evaluation of $S = S(\dot{L})$ by calculus is necessary as $L = L(\dot{L})$ cannot be solved explicitly. In fact, it is easier to evaluate $S = S(\dot{L})$ directly even for the logistic growth equation, as is done below.

© 1996 NRC Canada

Fig. 1. Relationships between otolith size S and somatic growth rate $\dot{L}$ for a hypothetical fish, with $L_\infty = 98.7$ cm, $K = 0.18 \cdot yr^{-1}$, $a = 0.000\ 117\ g \cdot cm^{-b}$, $b = 2.272\ 330$, and $c = -0.006\ 389$ g under the assumption that $S = S(t,L)$ is given by the general allometric equation and $L = L(t)$ by the von Bertalanffy (a), logistic (b), and Gompertz (c) growth equations.

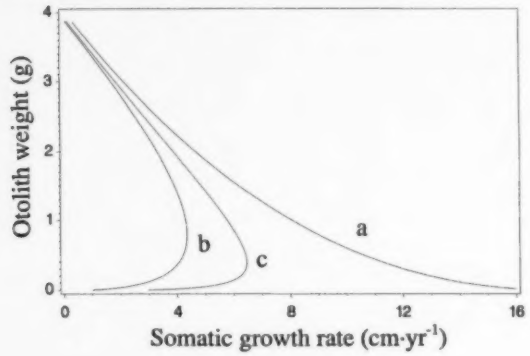
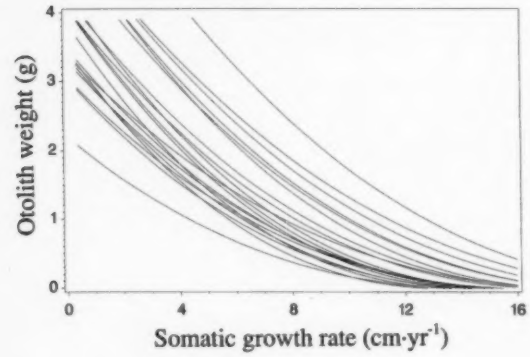
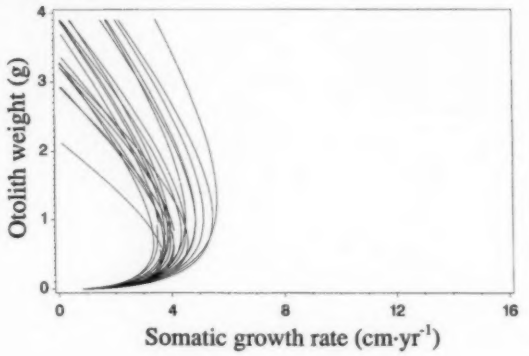


Fig. 2. Relationships between otolith size S and somatic growth rate $\dot{L}$ for a hypothetical group of fish, with $\bar{L}_\infty = 98.7$ cm, $\sigma^2_{L_\infty} = 9.87^2$ cm^2, $\bar{K} = 0.18 \cdot yr^{-1}$, $\sigma^2_K = 0.018^2 \cdot yr^{-2}$, $a = 0.000\ 117\ g \cdot cm^{-b}$, $b = 2.272\ 330$, and $c = -0.006\ 389$ g, under the assumption that $S = S(t,L)$ is given by the general allometric equation and $L = L(t)$ by the von Bertalanffy growth equation.



Equation 8.0 or 8.1 or that derived for the Gompertz growth equation applies to only a single individual (or a group of individuals with an identical set of somatic and otolith growth parameters). These equations are used in a simulation study of otolith size as a function of somatic growth rate for different individuals with different growth parameters, where I assume, for illustration, that a, b, and c are all constant and that K and L_∞ follow the joint normal distribution, i.e., $(K, L_\infty) \sim N(\bar{K}, \bar{L}_\infty; \sigma^2_K, \sigma^2_{L_\infty})$, with means $\bar{L}_\infty$ and $\bar{K}$ and variances σ^2_K and $\sigma^2_{L_\infty}$, although any other reasonable assumption can be made. For clarity in presentation, I simulate only 20 sets of data for a hypothetical group of fish, with $\bar{L}_\infty = 98.7$ cm, $\sigma^2_{L_\infty} = 9.87^2$ cm^2, $\bar{K} = 0.18 \cdot yr^{-1}$, $\sigma^2_K = 0.018^2 \cdot yr^{-2}$, $a = 0.000\ 117\ g \cdot cm^{-b}$, $b = 2.272\ 330$, and $c = -0.006\ 389$ g. The simulated data are then plotted to visualize the empirical relationship between otolith size and somatic growth rate.

Fig. 3. Relationships between otolith size S and somatic growth rate $\dot{L}$ for a hypothetical group of fish, with $\bar{L}_\infty = 98.7$ cm, $\sigma^2_{L_\infty} = 9.87^2$ cm^2, $\bar{K} = 0.18 \cdot yr^{-1}$, $\sigma^2_K = 0.018^2 \cdot yr^{-2}$, $a = 0.000\ 117\ g \cdot cm^{-b}$, $b = 2.272\ 330$, and $c = -0.006\ 389$ g, under the assumption that $S = S(t,L)$ is given by the general allometric equation and $L = L(t)$ by the logistic growth equation.



Results

I now examine, analytically, a special case of eqs. 8.0 and 8.1 and their equivalent for the Gompertz growth equation for constant a, b, and c. Somatic growth rate affects otolith size through allometric parameters or, in general, parameters in $S = S(t,L)$ and somatic growth parameters. In a single individual (or a group of individuals with an identical set of independent growth and allometric parameters) of a species of fish whose growth follows von Bertalanffy equation, its otolith size decreases with $\dot{L}$ ($\partial S/\partial \dot{L} = -ab/K(L_\infty - \dot{L}/K)^{b-1} < 0$) (Fig. 1$a$). In an individual whose growth follows the logistic equation, its otolith size increases with $\dot{L}$ ($\partial S/\partial \dot{L} = abL_\infty L^{b-1}/(K(L_\infty - 2L)) > 0$) if $L < L_\infty/2$ and decreases with it ($\partial S/\partial \dot{L} = abL_\infty L^{b-1}/(K(L_\infty - 2L)) > 0$) if $L > L_\infty/2$, and equals $c + a(L_\infty/2)^b$ ($\partial S/\partial \dot{L} = abL_\infty L^{b-1}/(K(L_\infty - 2L)) \rightarrow \infty$) if $L = L_\infty/2$ (Fig. 1b). In an individual whose growth follows the Gompertz equation, its otolith size increases with $\dot{L}$ ($\partial S/\partial \dot{L} = abL^{b-1}/(K(\log_e(L_\infty/L) - 1)) > 0$) if $L < L_\infty/e$ and decreases with it ($\partial S/\partial \dot{L} = abL^{b-1}/(K(\log_e(L_\infty/L) - 1)) < 0$) if $L > L_\infty/e$, and equals $c + a(L_\infty/e)^b$ ($\partial S/\partial \dot{L} = abL^{b-1}/(K(\log_e(L_\infty/L) - 1)) \rightarrow \infty$) if $L = L_\infty/e$ (Fig. 1c).

A family of relationships of $S = S(\dot{L})$ results if K and L_∞ vary among individuals singly or in combination. For the von Bertalanffy equation, if allometric parameters are independent of somatic growth parameters, S increases with K ($\partial S/\partial \dot{L} = abL/K^2(L_\infty - \dot{L}/K)^{b-1} > 0$) or L_∞ ($\partial S/\partial L_\infty = ab(L_\infty - \dot{L}/K)^{b-1} > 0$) if the other is fixed; S may increase or decrease with K and L_∞ relative to other paired values of K and L_∞, if K and L_∞ vary simultaneously (Fig. 2).

For the logistic equation, if $L < L_\infty/2$, S increases with either K ($\partial S/\partial K = abL^b(L_\infty - L)/(K(L_\infty - 2L)) > 0$) or L_∞ ($\partial S/\partial L_\infty = abL^{b+1}/(L_\infty(L_\infty - 2L)) > 0$) if the other is fixed; S may decrease or increase with K and L_∞ if K and L_∞ vary simultaneously, whereas if $L > L_\infty/2$, S decreases with either K ($\partial S/\partial K = abL^b(L_\infty - L)/(K(L_\infty - 2L)) < 0$) or L_∞ ($\partial S/\partial L_\infty = abL^{b+1}/(L_\infty(L_\infty - 2L)) < 0$), if the other is fixed; S may increase or decrease with K and L_∞, if K and L_∞ vary simultaneously (Fig. 3).

© 1996 NRC Canada

Fig. 4. Relationships between otolith size S and somatic growth rate $\dot{L}$ for a hypothetical group of fish, with $\bar{L}_\infty = 98.7$ cm^2, $\sigma^2_{L_\infty} = 9.87^2$ cm^2, $\bar{K} = 0.18 \cdot yr^{-1}$, $\sigma^2_K = 0.018^2 \cdot yr^{-2}$, $a = 0.000\,117$ g·cm^{-b}, $b = 2.272\,330$, and $c = -0.006\,389$ g, under the assumption that $S = S(t,L)$ is given by the general allometric equation and $L = L(t)$ by the Gompertz growth equation.

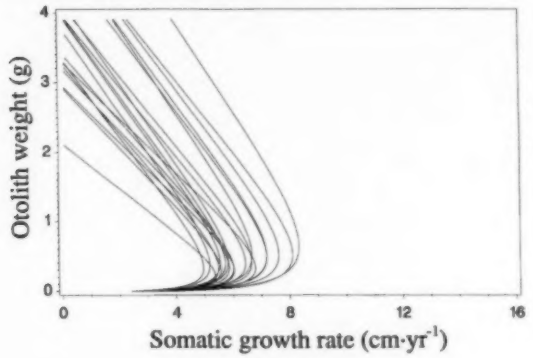
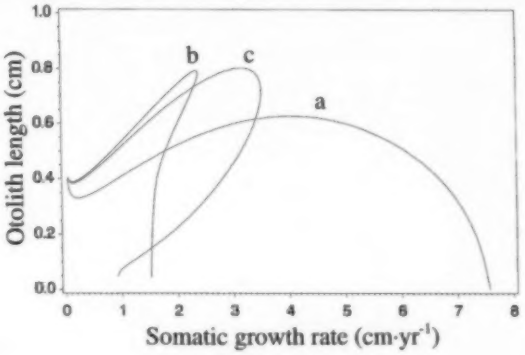


Fig. 5. Relationships between otolith length S and somatic growth rate $\dot{L}$ for the haddock (*Melanogrammus aeglefinus*), with $L_\infty = 61.94$ cm, $K = 0.155 \cdot yr^{-1}$, and $t_0 = -1.23$ yr for the von Bertalanffy (a, $L(t_0) = 0$), logistic (b, $L(t_0) = 10$ cm), and Gompertz (c, $L(t_0) = 0.7$ cm) growth equations. These estimates of L_∞, K, and t_0 are derived by averaging their estimates for 1951–1955 (64.77 cm, 0.15·yr^{-1}, –1.23 yr) and for 1956–1960 (59.11 cm, 0.16·yr^{-1}, –1.23 yr) (Templeman et al. 1978). Notice that these curves are not on the same scale and are only to show their general trends.



For the Gompertz equation, if $L < L_\infty/e$, S increases with either K ($\partial S/\partial K = abL^b/(K(\log_e(L_\infty/(L) - 1))\log_e(L_\infty/L)) > 0$) or L_∞ ($\partial S/\partial L_\infty = abL^b/(L_\infty(\log_e(L_\infty/L) - 1)) > 0$) if the other is fixed; S may decrease or increase with K and L_∞ if K and L_∞ vary simultaneously, whereas if $L > L_\infty/e$, S decreases with either K ($\partial S/\partial K = abL^b/(K(\log_e(L_\infty/L) - 1))\log_e(L_\infty/L) < 0$) or L_∞ ($\partial S/\partial L_\infty = abL^b/(L_\infty(\log_e(L_\infty/L) - 1)) < 0$), if the other is fixed; S may increase or decrease with K and L_∞, if K and L_∞ vary simultaneously (Fig. 4).

In their general form, eqs. 8.0 and 8.1 and their equivalent for the Gompertz growth equation have three unspecified functions. Instead of evaluating them, I now analyse, as a case study, Templeman and Squires' (1956) observation in the haddock (*Melanogrammus aeglefinus*) that the ratio of the length and weight of its otolith to its total length decreased approximately linearly with its total length for a given age but increased with its age for a given total length. Similar observations and inferences have also been made by Reznick et al. (1989) and Secor and Dean (1989, 1992). Such an approximately linear decrease in the ratio of haddock otolith length to its total length with an increase in its total length for a given age is translatable into $S/L = a(t) + b(t)L$ or $S = (a(t) + b(t)L)L$, with an intercept of $a(t) \geq 0$ and a slope of $b(t) \leq 0$ for a fish of age t: $a(3) = 0.048\,763\,6$, $a(6) = 0.049\,818\,2$, and $a(12) = 0.050\,454\,5$; $b(3) = -9.6050 \times 10^{-4} \cdot cm^{-1}$, $b(6) = -8.4109 \times 10^{-4} \cdot cm^{-1}$, and $b(12) = -7.8552 \times 10^{-4} \cdot cm^{-1}$. A plot of these digitized (from their Fig. 2) intercepts and slopes on age t indicates that both are described very well by a function of the form $f(t) = \gamma + \alpha t^\beta$, where α, β, and γ are parameters to be computed, with $a(t) = 0.043\,321 + 0.004\,520\ t^{0.1862}$ and $b(t) = -7.3716 - 7.5081\ t^{-1.1036} \cdot cm^{-1}$. Thus, $S = (0.043\,321 + 0.004\,520\ t^{0.1862} - (7.3716 + 7.5081\ t^{-1.1036})L)L$ cm. Successively substituting eqs. 6.0 and 7.0 into this equation yields

$$(9.0)\quad S = \left(0.043\,321 + 0.004\,520\left(t_0 - \frac{1}{K}\log\left(\frac{\dot{L}}{K(L_\infty - L(t_0))}\right)\right)^{0.1862} - \left(7.3716 + 7.5081\left(t_0 - \frac{1}{K}\log\left(\frac{\dot{L}}{K(L_\infty - L(t_0))}\right)\right)^{-0.1862}\right) \times \left(L_\infty - \frac{\dot{L}}{K}\right)\right)\left(L_\infty - \frac{\dot{L}}{K}\right)$$

for a fish whose growth follows the von Bertalanffy equation; successively substituting eqs. 6.1 and 7.1 into this equation gives

$$(9.1)\quad S = \left(0.043\,321 + 0.004\,520\left(t_0 - \frac{1}{K}\log\left(\frac{L(t_0)\left(1 \mp \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}{(L_\infty - L(t_0))\left(1 \pm \left(1 - \frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}\right)\right)^{0.1862}\right.$$

© 1996 NRC Canada

$$-\left(7.3716+7.5081\left(t_0-\frac{1}{K}\log\left(\frac{L(t_0)\left(1\mp\left(1-\frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}{(L_\infty-L(t_0))\left(1\pm\left(1-\frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)}\right)\right)^{-1.1036}\right)$$

$$\times\left(\frac{L_\infty}{2}\left(1\pm\left(1-\frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)\right)\Bigg/\left(\frac{L_\infty}{2}\left(1\pm\left(1-\frac{4\dot{L}}{KL_\infty}\right)^{1/2}\right)\right)$$

for a fish whose growth follows the logistic equation. $S = S(L)$ cannot be written in closed form for a fish whose growth follows the Gompertz growth equation. Equations 9.0 and 9.1 and their equivalent for the Gompertz growth equation show some really interesting trends (Fig. 5), which may suggest either a new pattern of fish otolith length as a function of their total length and age, or an improper formulation of $S = (a(t) + b(t)L)L$ as the otolith length of haddock as a function of its total length and age.

Discussion

This work demonstrates, with examples, that direct relationships between otolith size and somatic growth rate can be derived for a single individual or for a group of individuals with an identical set of growth parameters from known somatic growth equations and equations relating the size of that hard part to somatic size. It also provides an alternative approach to experimental study of otolith size as a function of somatic growth rate in individuals with different growth parameters. Although intended specifically for fishes, the argument applies equally to a great variety of animals and a great diversity of biological phenomena, such as allometric relations (Peters 1983; Calder 1984; Schmidt-Nielsen 1984; Reiss 1989). It also develops specific models (eqs. 2.0–8.1); other specific models can be derived easily.

The models formulated above have many other applications. An obvious one would be to improve experimental designs. It is dangerous to assume that early life history stages always grow faster than later ones, as is the case with animals whose growth adheres strictly to the von Bertalanffy equation, where $\dot{L}$ decreases linearly with L from KL_∞ at $L = 0$ to zero at $L = L_\infty$. It does not apply to animals whose growth follows the logistic equation, where L quadratically increases with L from zero at $L = 0$ to a maximum, $(1/4)KL_\infty$, at $L = (1/2)L_\infty$, and then decreases to zero at $L = L_\infty$. Nor does it apply to animals following the Gompertz equation, where L increases nonlinearly with $\dot{L}$ from zero at $L = 0$ to a maximum, KL_∞/e, at $L = L_\infty/e$, and then decreases to zero at $L = L_\infty$. Thus, animals at later life history stages may not necessarily grow more slowly than those at early stages. Caution should also be exercised in designing experiments on otolith growth and somatic growth rate in fishes, because the effects of somatic growth rate on fish otolith size might not be detectable for too narrow an experimental range of growth rates. Finally, it might be interesting to note that, for constant allometric parameters, an increase of otolith size with somatic growth rate, as predicted by the logistic and Gompertz equations for fish larvae and juveniles, remains to be experimentally verified. Since most previous studies have been directed at those life history stages, does this mean that the studied fish do not follow the logistic or Gompertz growth equation?

Findings of this study may help to correct for biases in back-calculated fish lengths at age. There are, however, some practical problems with making inferences from experimental observations in this context, because length-dependent mortality, biased sampling, faulty back-calculation, incorrect ageing, and differing somatic growth rate are all possible contributors to biases (Duncan 1980; Campana 1990). Also, individual variability in and correlation between growth parameters, ageing errors, sampling biases, and (or) other factors must be considered in growth analysis (Sainsbury 1980; Burr 1988; Francis 1988; Smith and McFarlane 1990; Hampton 1991; Xiao 1994; Xiao and Ramm 1994). Finally, although otoliths grow in both size and chemical composition, only their linear measurements have been used in growth studies, with a few exceptions (e.g., Maceina and Murphy 1989; Castonguay et al. 1991; Secor and Dean 1992; Smith 1992; Campana and Casselman 1993; Chauvelon and Bach 1993). Inappropriate sampling, faulty back-calculation, and differing somatic growth rates may well cause much fewer biases in back-calculation than this drastic dimensional reduction.

Reznick et al. (1989), Secor and Dean (1989, 1992), and Francis et al. (1993) reasoned that if slow-growing individuals have larger and heavier otoliths than fast-growing ones of the same length, then positive residuals of a linear regression of otolith weight (or length) on fish length will correspond to larger otoliths and lower somatic growth rates, while negative residuals will correspond to smaller otoliths and higher growth rates. In other words, residuals of such a regression would decrease with increasing somatic growth rate. The argument is sound, for if K and L_∞ are functions of fish age t and environmental factors (e.g., water temperature, and food quality and quantity), then otolith size also depends on fish age and environmental factors other than $\dot{L}$. However, to avoid possible biases in estimates of parameters from two separate regression

© 1996 NRC Canada

analyses, data could be fitted, directly, into eqs. 8.0 and 8.1 or their equivalents. Indeed, the success in deriving direct, analytical relationships between otolith size and somatic growth rate for a single individual (or a group of individuals with an identical set of somatic and otolith growth parameters) provides a basis for directly estimating all allometric parameters or in general parameters in $S = S(t,L)$ and somatic growth parameters from somatic growth rate and otolith size. Otolith size can then be used as a convenient index of somatic growth rate. However, such relationships can be very complex for different individuals with different growth rates (Figs. 2–4), and fall into the classic problem of errors in variables, where solutions are possible only under limited conditions. This may also require a large volume of data on fish otolith size and somatic growth rate, because of considerable parameter confounding. The requirement for data will be much reduced if some parameters, such as those in $S = S(t,L)$, are estimated externally. Finally, different functional forms of $S = S(t,L)$ may give different estimates of growth parameters. Therefore, it is essential that $S = S(t,L)$ be determined as accurately and precisely as possible.

There is a need to clarify terminology. An array of terms, including coupling (Wright 1990), uncoupling (Campana and Neilson 1985; Mosegaard et al. 1988; Secor and Dean 1989; Wright 1990; Francis et al. 1993), scaling (Reznick et al. 1989), and time lag (Molony and Choat 1990) have been used to describe the dependence of otolith size on somatic growth rate. Secor and Dean (1992) recognized this problem and believed that otolith growth continuously scales to somatic growth. As shown above, this dependence not only exists but can also be given, in some cases, in closed form for a single individual (or a group of individuals with an identical set of somatic and otolith growth parameters) and can be examined for individuals with different growth parameters. In the case of otolith size and somatic growth rate at least, these terms are inadequate for, and at worst misleading in, describing such dependence, resulting mainly from a lack of knowledge of the whole spectrum of growth rates. It is proposed here that they be abandoned and the term relationship be used to describe the dependence of otolith size on somatic growth rate.

Finally, verification of functional forms of relationships (for different individuals with different growth rates) between otolith growth rate and somatic growth rate, i.e., $\dot{S} = \dot{S}(\dot{L})$, would validate many applications of otolith microstructure studies (Campana and Neilson 1985). Much experimental work has been done to determine functional forms of $\dot{S} = \dot{S}(\dot{L})$(Geffen 1982; McGurk 1984; Neilson and Geen 1985; Secor et al. 1989), $R = R(\dot{L})$ (Secor et al. 1989), or $\dot{S} = \dot{S}(L)$ (Mosegaard et al. 1988). "Despite 20 yr of concerted effort, investigators have not been able to quantify a comprehensive or accurate relationship," according to Secor and Dean (1992). However, like $S = S(\dot{L})$, this relationship is readily deducible for a single individual or a group of individuals with an identical set of growth parameters. An example for $\dot{S} = \dot{S}(L)$ would suffice. Let $S = S(t,L)$ and $L = L(t)$ be given by eqs. 2.0 and 3.0, respectively. Substitution of eq. 7.0 into the first derivative of $S = S(t,L)$ with respect to L gives $\dot{S} = abL^{b-1}\dot{L} = ab(L_\infty - L/K)^{b-1}\dot{L}$. Similarly, individually varying growth parameters can be examined; I leave this and others to the interested readers to investigate.

Acknowledgments

I am indebted to Dr. David H. Secor of the University of Maryland for his valuable and constructive criticisms. I thank Drs. You-Gan Wang and Vivienne Mawson of CSIRO Division of Fisheries for commenting on the revised manuscript and an anonymous referee for useful criticisms. Drs. Chris Francis and Malcolm P. Francis of the Fisheries Research Center of the New Zealand Ministry of Agriculture & Fisheries, Richard C. Willan of the Northern Territory Museum of Arts and Sciences, and Brett W. Molony of James Cook University made useful comments on an earlier version of the manuscript. Drs. Malcolm P. Francis and Brett W. Molony generously supplied data for examination.

References

Burr, R.L. 1988. Inferring the distribution of the parameters of the von Bertalanffy growth model from length moments. Can. J. Fish. Aquat. Sci. **45**: 1779–1788.

Calder, W.A. 1984. Size, function, and life history. Harvard University Press, Cambridge, Mass.

Campana, S.E. 1990. How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. **47**: 2219–2227.

Campana, S.E., and Casselman, J.M. 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. **50**: 1062–1083.

Campana, S.E., and Neilson, J.D. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. **42**: 1014–1032.

Casselman, J.M. 1990. Growth and relative size of calcified structures of fish. Trans. Am. Fish. Soc. **119**: 673–688.

Castonguay, M., Simard, P., and Gagnon, P. 1991. Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. Can. J. Fish. Aquat. Sci. **48**: 296–302.

Chauvelon, P., and Bach, P. 1993. Modelling the otolith shape as an ellipse: an attempt for back-calculation purposes. ICES J. Mar. Sci. **50**: 121–128.

Duncan, K.W. 1980. On the back-calculation of fish lengths; modifications and extensions to the Frazer–Lee equation. J. Fish Biol. **16**: 725–730.

Francis, M.P., Williams, M.W., Pryce, A.C., Pollard, S., and Scott, S.G. 1993. Uncoupling of otolith and somatic growth in *Pagrus auratus* (Sparidae). Fish. Bull. **91**: 159–164.

Francis, R.I.C.C. 1988. Are growth parameters estimated from tagging and age-length data comparable? Can. J. Fish. Aquat. Sci. **45**: 936–942.

Geffen, A.J. 1982. Otolith ring deposition in relation to growth rate in herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. Mar. Biol. (Berlin), **71**: 317–326.

Hampton, J. 1991. Estimation of southern bluefin tuna *Thunnus maccoyii* growth parameters from tagging data, using von Bertalanffy models incorporating individual variation. Fish. Bull. **89**: 577–590.

Huxley, J.S. 1972. Problems of relative growth. 2nd ed. Dover Publications, Inc., New York.

Maceina, M.J., and Murphy, B.R. 1989. Differences in otolith morphology among the two subspecies of largemouth bass and their F_1 hybrid. Trans. Am. Fish. Soc. **118**: 573–575.

Marshall, S.L., and Parker, S.S. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Can. J. Fish. Aquat. Sci. **39**: 542–547.

McGurk, M.D. 1984. Ring deposition in the otoliths of larval Pacific herring, *Clupea harengus pallasi*. Fish. Bull. **82**: 113–120.

Molony, B.W., and Choat, J.H. 1990. Otolith increment widths and

© 1996 NRC Canada

somatic growth rate: the presence of a time-lag. J. Fish Biol. **37**: 541–551.

Mosegaard, H., Svedäng, H., and Taberman, K. 1988. Uncoupling of somatic and otolith growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. Can. J. Fish. Aquat. Sci. **45**: 1514–1524.

Neilson, J.D., and Geen, G.H. 1985. Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fish. Bull. **83**: 91–101.

Peters, R.E. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Reiss, M.J. 1989. The allometry of growth and reproduction. Cambridge University Press, Cambridge.

Reznick, D., Lindbeck, E., and Bryga, H. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci. **46**: 108–112.

Sainsbury, K.J. 1980. Effect of individual variability on the von Bertalanffy growth equation. Can. J. Fish. Aquat. Sci. **37**: 241–247.

Schmidt-Nielsen, K. 1984. Scaling: why is animal size so important? Cambridge University Press, Cambridge, New York, New Rochelle, Melbourne and Sydney.

Schnute, J. 1981. A versatile growth model with statistically stable parameters. Can. J. Fish. Aquat. Sci. **38**: 1128–1140.

Secor, D.H., and Dean, J.M. 1989. Somatic growth effects on the otolith – fish size relationship in young pond-reared striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. **46**: 113–121.

Secor, D.H., and Dean, J.M. 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. **49**: 1439–1454.

Secor, D.H., Dean, J.M., and Baldevarona, R.B. 1989. Comparison of otolith growth and somatic growth in larval and juvenile fishes based on otolith length/fish length relationships. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **191**: 431–438.

Smith, D.S., and McFarlane, G.A. 1990. Growth analysis of Strait of Georgia lingcod by use of length-frequency and length-increment data in combination. Trans. Am. Fish. Soc. **119**: 802–812.

Smith, M.K. 1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. Can. J. Fish. Aquat. Sci. **49**: 795–804.

Templeman, W., and Squires, H.J. 1956. Relationship of otolith lengths and weights in the haddock *Melanogrammus aeglefinus* (L.) to the rate of growth of the fish. J. Fish. Res. Board Can. **13**: 467–487.

Templeman, W., Hodder, V.M., and Wells, R. 1978. Age, growth, year-class strength, and mortality of the haddock, *Melanogrammus aeglefinus*, on the southern Grand Bank and their relation to the haddock fishery of this area. Int. Comm. Northwest Atl. Fish. Res. Bull. No. 13. pp. 31–52.

Wright, P.J., Metcalfe, N.B., and Thorpe, J.E. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L: evidence against coupling. J. Fish Biol. **36**: 241–249.

Xiao, Y. 1994. von Bertalanffy growth models with variability in, and correlation between, K and L. Can. J. Fish. Aquat. Sci. **51**: 1585–1590.

Xiao, Y., and Ramm, D.C. 1994. A simple generalized model of allometry, with examples of length and weight relationships for 14 species of groundfish. Fish. Bull. **92**: 664–670.

© 1996 NRC Canada

PERSPECTIVE

Population stewardship rights: decentralized management through explicit accounting of the value of uncaught fish

Stratis Gavaris

Abstract: Fisheries management in Atlantic Canada has typically involved harvest control by a central authority. There is increasing recognition for the merits of enhanced participation by fishers in the decision-making process. To facilitate implementation of decentralized management, a population stewardship rights fishery system is proposed that explicitly recognizes the value of fish remaining in the water, offers a mechanism for partitioning that value among elementary management units (EMUs), and uses established fisheries dynamics for feedback and accountability on the consequences of actions. In a population stewardship rights fishery, the fish stock is partitioned into conceptual population shares that are entrusted to the care of individual or groups of fishers operating under common rules, the EMUs. EMUs are shielded from the consequences of other's actions by counting the catch by each EMU against their own partial population. Furthermore, EMUs earn a share of recruiting year-classes according to the relative magnitude of the spawning potential of their partial population, which reflects their success at stewardship of the population share entrusted to their care. Portions of recruiting year-classes may be reserved by central authorities to meet conservation objectives if the actions of EMUs threaten the productive capacity of the resource.

Résumé : La gestion des pêches dans la région du Canada atlantique passe généralement par un contrôle des captures effectué par une autorité centrale. On reconnaît toutefois de plus en plus l'intérêt d'accroître la participation des pêcheurs au processus décisionnel. Pour faciliter la mise en place d'une gestion décentralisée, nous proposons un système de pêche fondé sur des droits de gérance des populations, qui reconnaît explicitement la valeur des poissons demeurés dans l'eau, offre un mécanisme de répartition de cette valeur entre des unités élémentaires de gestion (UEM) et se fonde sur la dynamique des pêches établies pour assurer la rétroaction et la responsabilisation quant aux conséquences des actes. Dans une pêche fondée sur les droits de gérance des populations, le stock de poisson est réparti en parts théoriques de population qui sont confiées aux soins de pêcheurs ou de groupes de pêcheurs fonctionnant selon des règles communes, les UEM. Les UEM sont protégées des conséquences des actes des autres du fait que chacune mesure ses prises par rapport à sa propre portion de la population. De plus, les UEM gagnent une part des classes recrutées à la pêche en fonction de la grandeur relative du potentiel de reproduction de leur portion de la population, ce qui traduit le succès obtenu dans la gérance de la part de population qui leur a été confiée. Certaines portions des classes recrutées à la pêche peuvent être réservées par les autorités centrales en vue d'atteindre les objectifs de conservation si les actes des UEM menacent la capacité de production de la ressource.
[Traduit par la Rédaction]

Introduction

Recent severe declines of traditional groundfish resources in Atlantic Canada have prompted examination of how existing fishery management systems performed. The reasons for failing to achieve the expected benefits from these fishery resources are complex and interrelated and it is unlikely that a consensus on a simple answer will emerge. A review of groundfish resource management in the Scotia–Fundy region concluded that the objectives were sound, the strategy was appropriate, and the available information was adequate but that the tactical approach chosen did not control fishing mortality and generated illegal behavior, which was not curbed by the available enforcement regime (Angel et al. 1994). The best use was not made of available knowledge, however imperfect or incomplete that knowledge may have been. For example, despite possible deficiencies in the scientific advice, the declining trends had been identified and warnings of excessive exploitation had been given (Canadian Atlantic Fisheries Scientific Advisory Committee 1993), yet there continued to be pressures for higher total allowable catches.

In Atlantic Canada, groundfish management has principally involved harvest control in the form of quotas but has also included a variety of other measures such as limited entry licensing, vessel restrictions, gear restrictions, fish size limits, and area or season closures. The Department of Fisheries and Oceans took a top-down approach to governing fisheries rather than building on the available social capital represented by

Received June 26, 1995. Accepted January 19, 1996.
J12972

S. Gavaris. Department of Fisheries and Oceans, Biological Station, St. Andrews, NB E0G 2X0, Canada. e-mail: sgavaris.sta.dfo.ca

© 1996 NRC Canada

local institutions in a bottom-up fashion (Ostrom 1990). The process for development of a groundfish management plan was highly centralized and based on the ultimate authority of the Department. There was limited scope and flexibility to accommodate local circumstances or to permit diverse implementations of the strategy.

Until recently, global quotas were set for stocks, and fishers competed for the catch until these overall quotas were reached. Granting of secure annual harvest rights to enterprises for defined portions of the overall quotas, referred to as enterprise or individual quotas, were first introduced for the offshore fleets during the mid-1980s and then for inshore draggers during the early 1990s and gave these users more say over their affairs. Fishers, however, continued to criticize their limited influence over fisheries management. While fishers are seeking meaningful participation and a greater role in fisheries management, the Department of Fisheries and Oceans is also re-examining its mandate. The Department continues to recognize as its prime responsibility, ensuring the conservation of resources, while acknowledging the merit of involving fishers in finding solutions for optimizing the socioeconomic objectives. Because the conservation and socioeconomic objectives are interdependent, however, a co-operative partnership between the Department and fishers will need to be developed.

A less authoritative role by the Department of Fisheries and Oceans in the fisheries management process is likely to accentuate the heterogeneity between fishers with diverse interests and incompatible and often poorly specified objectives, and the absence of a common ground to resolve differences. A less centralized approach to fisheries management will require the establishment of effective procedures for reaching consensus and (or) the capability to accommodate diverse approaches by fishers. Uniformity need not be imposed. Where the benefits of reaching consensus on consistent practices outweigh the costs of negotiating such common rules, uniformity may prevail; otherwise, a diverse approach may be more practical. To permit local diversity, though, decisions made by resource users must have a limited impact on others. The orientation towards decentralized management is consistent with current trends in public policy. Centralized resource management is founded on the premise that the required knowledge can be concentrated amongst a few informed experts: scientists, economists, politicians, etc. An emerging alternative paradigm considers the requisite information to be diffuse, thereby necessitating local involvement in management decisions (Anderson and Leal 1991).

Past emphasis on centralized fisheries management has influenced the development of the scientific models used to aid the decision-making process. Traditional fishery science is largely aimed at providing uniform advice for geographically expansive management units (Canadian Atlantic Fisheries Scientific Advisory Committee 1993). Here, I develop one approach for accommodating decentralized fisheries management. The proposed system aims to minimize the need for uniformity amongst fishers by limiting the impact of decisions by resource users on others. It allows reduced central regulation but relies on effective monitoring and on incorporation of feedback mechanisms for the consequences of actions taken by fishers.

Population stewardship rights fishery system

The classical notion of a management unit implies that decisions are made at that one scale. Here, management units are viewed as a nested or hierarchical structure, some decisions being made at local scales whereas others are negotiated consensus agreements over broad geographic areas. I refer to an individual fisher or any group of fishers operating under common rules as an elementary management unit (EMU). One way to accommodate local participation in fisheries management is to parcel the resource among users. This requires explicit recognition that uncaught fish have value and to partition that value into EMUs.

Quota-based fishery management systems are inappropriately founded on attributing value only to caught fish. With enterprise or individual quotas, where the privilege to catch fish is secured at the beginning of the fishing season, that prospective value has even manifested itself in the trade and sale of quota before the fish are caught. However, even with these, it is only fish that have been allocated that are attributed value. Fish remaining in the water also have value, though, referred to as the natural capital by ecological economists (Rees 1991). Rational fisheries management systems should find a way to reflect the value of this natural capital.

The value of uncaught fish is related to size and age. Fish typically experience larger growth increments at younger ages (Ricker 1975); therefore, the net production of fish, considering gains from somatic growth and losses from natural mortality, differs between younger fish and older fish. Also, fecundity is generally related to weight (Beverton and Holt 1957) and maturity is length dependent; therefore, the contribution by small fish to reproduction is not the same as that of larger fish. Age-based accounting allows us to properly measure the natural capital represented by uncaught fish.

The notion of attributing value to uncaught fish is appreciated by fishers and was exemplified by inquiries from a Grand Manan, New Brunswick, fisher shortly after the introduction of individual quotas in his area. He, like others in that area, fished both groundfish and lobster, incurring an expense to modify his boat each time he switched. Since his annual groundfish quota was small, he asked about carrying it over from one year to the next. However, he also wanted to augment his quota by the amount those fish would grow while they were left in the water and by the amount their offspring would contribute. He accepted the loss of some fish from natural deaths. These proposals were similar in principle to bankable quotas (Townsend 1992).

This fisher's request could not be granted within the existing management system, in part because its impact on the opportunities of others could not be assessed. Existing systems have limited accommodation for independent action, making them unnecessarily dependent on establishment of common rules for virtually all aspects of fishing activity over very large spatial scales. The rationale for this approach is based on biological considerations. Fish stocks are generally defined as self-sustaining biological populations. Fish movement within stock boundaries generally precludes definition of meaningful physical boundaries at a scale smaller than a stock. Consequently, stock status must be evaluated at this broad spatial scale. Traditional fishery systems have tacitly adopted this

© 1996 NRC Canada

biological scale as the management unit, the scale at which governing institutions were established. In the Scotia–Fundy region, for example, industry–government advisory committees had been established to coincide with principal groundfish stock areas but were abandoned when they proved ineffective. The large number of divergent interests involved in Northwest Atlantic groundfish fisheries, coupled with the broad distributional range of many stocks, created a debilitating mismatch in the scale at which scientific advice was provided and the scale at which governing institutions could be effective. Many fishers felt alienated by the process and were frustrated by their exclusion from meaningful participation in management decisions that affected their livelihood.

Reaching consensus on harvest strategies and management tactics for an entire stock has the advantage of consistency, but often even compromises are difficult to achieve with the diversity of interests involved in fisheries. Common general objectives such as optimizing yield or profit translate into divergent operational goals. A situation occurring in the Canadian fishery on Georges Bank demonstrates this. One resource user had a limited interest in haddock, *Melanogrammus aeglefinus*, but conducted a directed fishery for pollock, *Pollachius virens*, in which some small haddock were taken as a by-catch. It was important to maintain a sufficient haddock allotment to permit them to continue a pollock fishery. The size of the haddock they caught was not of paramount importance. This was very different from the aim of other fishers that targeted haddock and sought to catch large fish, which had higher value. Even in this simple situation, a common harvest strategy would probably not be optimal for all users.

The population stewardship rights fishery system exploits the possibility of monitoring a conceptual partial population, that portion of a population endowed to the care of a fisher or a group of like-minded fishers and identified as an EMU. This achieves the objective of attributing value to uncaught fish and partitioning that value in a manner that limits the impact of decisions by an EMU on others. By effectively shielding EMUs, the population stewardship rights fishery can accommodate a greater degree of independent action and facilitate the participation of fishers in the management process at the local EMU scale.

Conceptual partial population

The capability to attribute value to fish remaining in the water and to partition that value is accommodated by the computation of conceptual partial populations. Consider one year-class. The partial population abundance at age 1 in year y for EMU e is the total population abundance at age 1, $N_{1,y}$, estimated from the most recent assessment, multiplied by that EMU's share of that year-class, $P_{e,1,y}$:

$$N_{e,1,y} = P_{e,1,y} N_{1,y}$$

The abundance of that EMU's conceptual share of this year-class is diminished each year by the number that died from natural mortality and the number caught by that EMU. Therefore, the partial population abundance of that year-class for the EMU can be computed for any age a up to the current year by sequentially removing the catch by that EMU, $C_{e,a,y}$, and the natural deaths, $D_{e,a,y}$:

$$N_{e,a+1,y+1} = N_{e,a,y} - (C_{e,a,y} + D_{e,a,y})$$

The total stock natural deaths are partitioned among EMUs according to the magnitude of their partial populations:

$$D_{e,a,y} = D_{a,y}(N_{e,a,y}/N_{a,y})$$

Though a ratio of average abundance would be more consistent with population models for partitioning natural deaths among EMUs, it is simpler to use beginning of year abundance, and this provides an adequate approximation when exploitation rates do not differ greatly. The total stock natural deaths, $D_{a,y}$, are obtained from the stock assessment results:

$$D_{a,y} = M\bar{N}_{a,y}$$

where M is the natural mortality rate and $\bar{N}_{a,y}$ is the average stock abundance.

Doing this for all year-classes in the fishery and applying what we know about growth, we can determine the biomass of each EMU's conceptual partial population. A proxy to partial spawning biomass in any year for an EMU is obtained by summing the biomass of adult age groups

$$B_{e,y} = \sum_{a} N_{e,a,y} W_{a,y}$$

where $W_{a,y}$ is the weight at age of fish. Where reliable maturity schedules and (or) fecundity relationships are available, they can be applied to refine the measure of spawning potential.

Thus, we can partition an incoming year-class among the EMUs on the basis of the relative magnitudes of the adult biomass of their respective conceptual partial populations. Incoming recruitment is earned by each EMU according to their stewardship of the partial population endowed to their care. An EMU's share of a recruiting year-class, $P_{e,1,y}$, is set equal to the fraction of the total adult biomass contributed by that EMU's partial population in the year they were spawned:

$$P_{e,1,y} = B_{e,y-1}/B_{y-1}$$

It is possible for an EMU to exert a sufficiently high fishing mortality to exhaust their partial population of a year-class yet continue to capture fish of that year-class from the total population. That EMU is held accountable for the removed fish by inflating their removals from the next oldest age. This has the effect of appropriately accounting for the catch from that EMU and accordingly diminishing the respective conceptual partial population abundance. Say that $C_{e,10,y}$ is the number of age-10 fish caught by an EMU in excess of their partial share of that year-class. The adjusted catch at age 9 for that EMU, $C'_{e,9,y}$, would be calculated as

$$C'_{e,9,y} = C_{e,9,y} + C_{e,10,y} W_{10,y}/W_{9,y}$$

which augments the age-9 removals by the number equivalent to the excess biomass caught.

This bookkeeping of conceptual partial populations captures the fundamental processes of fisheries dynamics (recruitment, growth, and mortality) and facilitates partitioning the value of the natural capital at a scale smaller than a stock.

Deterministic simulation

A simple deterministic simulation of a population stewardship rights fishery is presented for illustrative purposes. A rigorous evaluation of performance for a management system based on

© 1996 NRC Canada

Table 1. Schedules of instantaneous fishing mortality rates for the deterministic simulation.

Age	1	2	3	4	5	6	7	8	9	10	11	12
EMU A	0	0.01	0.05	0.12	0.2	0.2	0.2	0.15	0.15	0.1	0.1	0.05
EMU B	0	0	0.025	0.1	0.175	0.245	0.25	0.25	0.25	0.25	0.25	0.25

Table 2. Rule governing number of recruits reserved in the deterministic simulation.

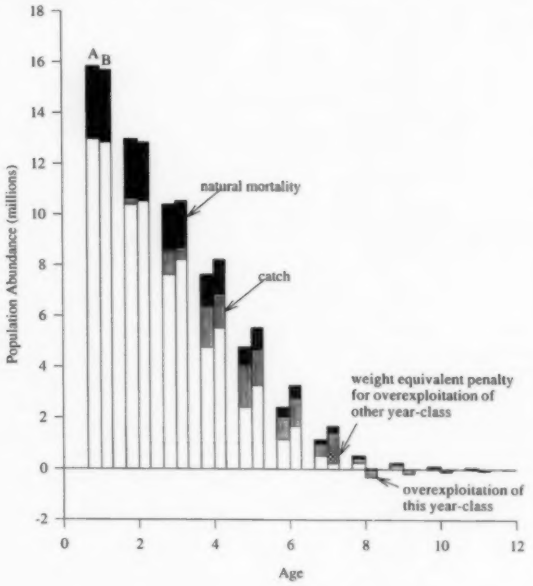
Biomass for ages 3 and older (t)	Recruits reserved
Greater than 60 000	0
Between 60 000 and 45 000	1 million
Between 45 000 and 30 000	2.5 million
Less than 30 000	5 million

these ideas is beyond the scope of this paper and would require detailed specification of the situation and the options being considered. More complete stochastic simulations could incorporate uncertainty in estimates of stock abundance and in the sampling error of the catch composition for the identified EMUs to examine issues related to precision of the perceived state parameters.

I constructed a hypothetical population with characteristics similar to those of Atlantic cod, *Gadus morhua*, in Division 4X of the Northwest Atlantic Fisheries Organization convention area. The starting population abundance in the 1st year of the simulations was similar to that observed during the late 1970s, and the abundance of recruiting year-classes corresponded to the observed series over the past two decades (Gavaris 1993). The growth and size at age were based on average results from Atlantic cod in Division 4X. The spawning biomass was approximated by the biomass for ages 3 and older. The example employed two EMUs, each with different exploitation patterns by age and different rates of fishing mortality on fish fully recruited to their fisheries. EMU A harvested fish at a younger age whereas EMU B exerted a higher instantaneous fishing mortality rate on adult fish according to the schedules in Table 1. In this simple example, neither EMU modified its behavior over the two decades that were simulated. Of course, in practice, EMUs could consider evolving conditions and evaluate various courses of action, including changes in strategy.

Assume that in the initial year, a conservation reserve was identified corresponding to a biomass for ages 3 and older of about 30 000 t, somewhat less than the lowest previously observed value from which the population had recovered. The age composition of the fish in the conservation segment was set to match that of a moderately fished (instantaneous fishing mortality rate of 0.2) population to ensure broad representation of age groups. The remaining population in that initial year was then partitioned equally among the two EMUs. Assume further that as each year-class recruited, a rule to reserve a portion for conservation purposes was implemented. The quantity of recruits reserved was dependent on the total biomass of adult fish present in the population at the beginning of that year. If the EMUs were collectively exerting a sufficiently moderate exploitation rate to maintain an adequate spawning potential, few or no recruits were reserved. If the fisheries were depleting the adult biomass, a greater amount was reserved. Historically, this population had average recruitment of about 20 million fish and the adult biomass had ranged between 45 000 and 90 000 t. The rule in Table 2 was used.

Fig. 1. EMU A began exploiting its share of this year-class at a younger age. EMU B, which exerted a higher exploitation rate at older ages, continued to catch fish from another year-class where its share had already been exhausted. Consequently, the weight equivalent for that overexploited quantity was charged against this year-class at age 7. EMU B subsequently depleted its share of this year-class by age 8 and has overexploited it as well.



The EMUs fished according to their desired exploitation pattern and their catch was counted against their respective conceptual partial populations. In each subsequent year, their conceptual partial population was comprised of those fish that they had not caught, diminished in number owing to natural deaths but accounting for increased weight from growth and augmented by that EMU's share of the new recruits. A comparison of how the two EMUs exploited the same year-class differently shows that EMU A depleted its population more quickly at a young age but EMU B went into a deficit when it was penalized for overexploitation of a previous year-class (Fig. 1). This demonstrates how an EMU that has caught more fish out of a year-class than they had remaining in their partial population was held accountable by having the equivalent weight in number removed from the next oldest available year-class that that EMU had not fished out. Excepting those recruits reserved for conservation when necessary, according to the rule described above, EMUs were allotted their share of recruits on the basis of the relative magnitude of the biomass of fish aged 3 and older in their respective conceptual partial

© 1996 NRC Canada

Fig. 2. The adult biomass of the conceptual partial population for EMU A was greater in early years, earning them more recruits. The exploitation rate exerted by EMU A, however, was sufficiently high at younger ages to result in a more rapid decline of its adult biomass and consequently its recruit endowment was reduced.

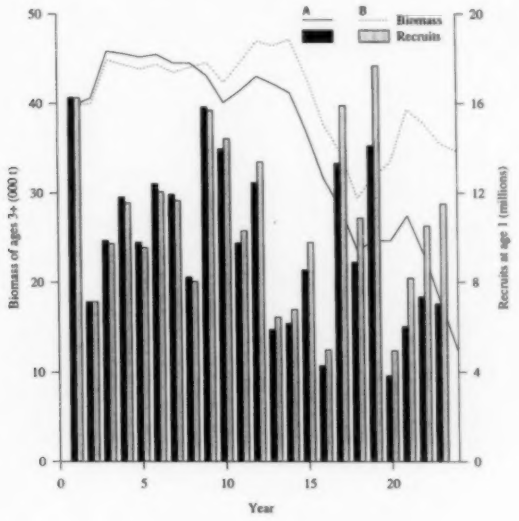
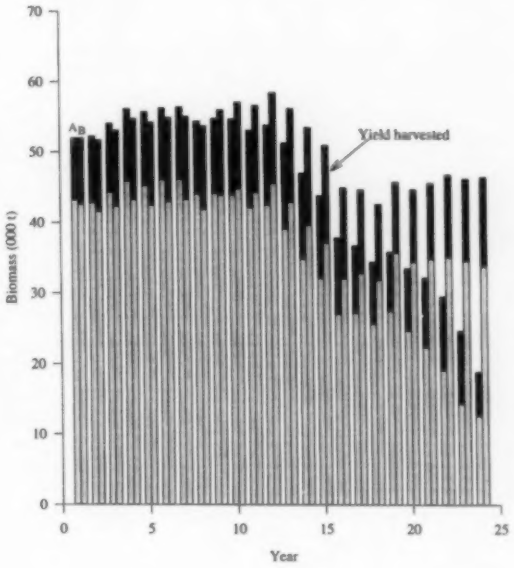


Fig. 3. EMU A exploited this resource more intensely at younger ages and suffered loss in yield from growth overfishing as well as a reduction of natural capital as the biomass of its conceptual partial population declined.



population. EMU A began depleting the adult biomass of its conceptual partial population more rapidly than EMU B following the 10th year and consequently, subsequent endowments of recruits to EMU A declined (Fig. 2). The yield that the EMUs extracted over the years was an outcome of their performance as stewards of the share of the resource that had been entrusted to them. The higher exploitation rate exerted by EMU A at younger ages eventually led to a decline of its partial population biomass and a concomitant reduction of yield (Fig. 3). Here I have simply used yield as a measure of performance but fishers may use their knowledge of economic factors to determine net earnings by accounting for the price paid them for the fish and expenses they incurred in harvesting, primary processing, and management activities.

Implications for management functions

Pinkerton and Weinstein (1995) divided management functions into seven general categories: (*i*) policy making and evaluation, (*ii*) ensuring productive capacity of the fishery, (*iii*) regulating access, (*iv*) regulating harvest, (*v*) resolving conflicting resource use and management, (*vi*) implementing rules, and (*vii*) optimizing benefits. A change to a population stewardship rights fishery system from the prevailing quota management approach can affect how activities associated with these functions are conducted. One aspect, which relates to all of these functions, is that local participation for many activities is facilitated. Harvest monitoring, resource monitoring, stock evaluation, and regulation of access and harvesting are activities considered further here because a change to population stewardship rights from existing quota systems could significantly affect not just how these are conducted but also what needs to be done in these areas.

Harvest monitoring for output-controlled systems such as quota-regulated fisheries is often achieved through reporting and observation of landings. This may be augmented by observation of at-sea activities to confirm that fishing activity and catches occurred in the reported areas and to check the degree of discarding of fish, if any. Since a population stewardship rights fishery is not output regulated, it may initially appear as though such monitoring is unnecessary. The computation of conceptual partial populations, however, relies on reliable information about the quantity of fish caught. Therefore, the requirements for monitoring landings and at-sea activities for population stewardship rights fisheries are comparable with those for quota-regulated fisheries, though there is no necessity to put in place regulatory systems that react to the amount caught.

The length composition of the catch must be monitored for quota-regulated fisheries to assess the condition of the stock. This is also the case for a population stewardship rights fishery, but in addition, information on length composition of the catch will also have to be used to evaluate the state of conceptual partial populations. Though conceptual partial population abundance can be computed with the data that are currently available, for stocks where analytical assessments are conducted, it has to be recognized that the precision of those estimates is influenced by the precision of the catch at age for that EMU. Since the precision of the catch at age is a function of the degree of sampling for size composition of the catch, it may be desirable to intensify the monitoring for size composition depending on the number of EMUs and the dissimilarities between their catches. In instances where it is reasonable to assume that the size composition of the catch by several EMUs is comparable, it may be adequate to use the pooled

© 1996 NRC Canada

Table 3. Comparison of requirements of a population stewardship rights system with those of quota fisheries.

Activity	Requirements for population stewardship rights compared with quota fisheries
Harvest monitoring	
Landings	Similar
At sea	Similar
Length composition of catch	Enhance depending on number of EMUs and on differences in catch
Resource monitoring	
Abundance indices	Similar
Resource evaluation	
Condition of stock	Similar
Status of conceptual partial populations	New function
Regulation	
Resource access	Rules limited to revoking fishing privileges when conceptual partial population is exhausted and to authority for transfer of population stewardship rights
Harvest control	Centralized regulations limited to conservation rules for recruitment overfishing

samples and thereby circumvent the need for additional sampling. Other techniques, such as grading of landings by size, e.g., small, market, and large, and using pooled samples within these categories, can also be employed to reduce the sampling requirements. It should be noted that additional information on size composition of catches can generally be obtained at relatively low cost compared with other information required for resource evaluation. Additional costs for enhanced monitoring should be weighed against increased benefits to EMUs from more reliable information on the status of conceptual partial populations.

The information obtained from monitoring the commercial fishery activity is typically augmented by information on trends in abundance to evaluate the condition of the stock. Traditional sources of abundance index time series include research surveys conducted by governments and catch rate information collected from the commercial fisheries. Both quota-regulated fisheries and population stewardship rights fisheries require quantitative estimates of the total stock and therefore make the same demands for abundance indices for stock monitoring and evaluation. Evaluation of the state of conceptual partial populations adds a new analytical and administrative load for population stewardship rights fisheries, but these calculations can be readily performed and tracked with the aid of computers.

Access to groundfish fisheries in Atlantic Canada involves both licensing and quota rules, but the two may not be complementary. This can result in a mismatch between harvesting capacity, which involves licensing, and the productivity of resources, which is expressed in the establishment of quotas. In a population stewardship rights fishery, regulation of access could be limited to revoking an EMU's fishing privileges if that EMU has exhausted their conceptual partial population. It becomes unnecessary to define distinct rules for licensing, quota distribution, or other conditions for fishing. Access rights become synonymous with stewardship rights for a conceptual partial population. As long as an EMU possesses population stewardship rights for a conceptual partial population, they have the right to harvest from it. If transfer of population stewardship rights are to be permitted between EMUs, rules to regulate such transfers should be considered.

Harvest control in Atlantic Canada involves numerous auxiliary regulations such as area or season closures, vessel size limits, gear specifications, and minimum fish size in addition to the principal tactic of catch quotas. By effectively limiting the impact of EMUs to their respective conceptual partial populations, centralized harvest regulation may be confined to the access considerations discussed above and to conservation concerns. Additional regulations may be added if users consider them useful and consensus is reached by all affected EMUs to introduce rules, but central governments need not be involved. An integral part of introducing rules, however, is ensuring that adequate enforcement is in place to maintain an acceptable level of compliance. A population stewardship rights fishery system operates between EMUs and is not affected by the use of rules or regulations deemed practical within EMUs.

Though population stewardship rights bestow EMUs with responsibility for maintaining the productive capacity of the resource, central governments generally assume the obligation for ensuring conservation of the resource and looking after the interests and opportunities of future generations. To fulfill their conservation mandate, government must consequently retain a role in establishing fishery monitoring standards that are sufficient for adequately evaluating the status of the resource. Additionally, though, they may establish conservation rules if the actions of EMUs are not adequately maintaining the productive capacity of the resource. Some studies of stock and recruit relationships suggest that production of recruits may decrease more rapidly when the spawning biomass falls below a point corresponding to half of the maximum recruitment on a theoretical stock and recruitment curve (Mace 1994; Myers et al. 1994). In the absence of convincing evidence for a stock and recruit relationship in any specific situation, consensus on arbitrary practical rules to help prevent recruitment overfishing, such as those used in the simulation example, may be sought.

Compared with existing groundfish quota management systems in Atlantic Canada, a population stewardship rights fishery system implies a shift towards enhanced monitoring activities and reduced centralized regulatory activities as summarized in Table 3.

© 1996 NRC Canada

Discussion

Recent debate on sustainable utilization of renewable resources has focused attention on the need for adequate consideration of the role of natural capital (Rees 1990, 1991). Successful management of natural resources can be compromised by ignoring the value of natural capital. Daly and Cobb (1989; see especially the appendix: The Index of Sustainable Economic Welfare) provide a comprehensive evaluation of measures of community welfare from this perspective and identify compelling arguments against basing wealth and well-being on only the rate of flow. Human capacity to utilize fisheries resources in a manner that depletes the natural capital suggests that yield is not a sufficient measure of stock welfare. Fisheries scientists have recognized this and past considerations in developing harvest strategies have included stock biomass. In this regard, the benefits of moderate exploitation rates, which include more stable yield, higher catch rates, which mean improved harvest efficiency, and larger average fish size, which usually brings better prices and better chances of good recruitment, are well recognized (Rivard et al. 1988). Yet despite the positive attributes associated with moderate exploitation, there appears to be a compelling drive to take more fish out of the water, depleting the natural capital. Fishers are displaying a rational response to an incentive system at odds with scientific knowledge, traditional wisdom, and ethical behavior. Existing quota management implementations fail to respect the value of the natural capital represented by uncaught fish in the water, and they create disincentives for the desired behavior by dissipating the consequences of detrimental actions among all users. Fishers' and managers' attention is largely focused on fluctuations in the magnitude of quotas with only a passing acknowledgment of changes to the stock abundance, the natural capital. Population stewardship rights provide one way to integrate explicit recognition of the natural capital of fish resources into the management process.

The current orientation towards co-management in fisheries places increased emphasis on local organization and governing institutions. Increased involvement of fishers and other stakeholders in the decision-making process will require careful consideration regarding the scale at which governing institutions are established. Some decisions may need to be made at fairly large scales, say Canadian Atlantic zone wide, while others would be better left to smaller more homogeneous units, say longline fishers in a particular county. The degree of difficulty in reaching consensus on issues is often related to the diversity of interests. Resolution of many issues and problems could be facilitated if smaller, like-minded groups could reach agreement independent of other fishery participants. In a nested system of governing institutions where umbrella organizations deal with increasingly broader issues, a useful principle is to delegate decision making to the smallest practicable scale, the scale at which all who are significantly affected are represented. It is necessary, then, that the decisions made at that scale should have a limited impact on others. By partitioning the natural capital, the stock of fish remaining in the water, among EMUs and effectively shielding the EMUs from the actions of others, the population stewardship rights fishery system substantially expands the scope for development of local governing institutions at scales smaller than the biological stock.

Introduction of a population stewardship rights system would require identification of suitable EMUs. Townsend (1995) advocated the implementation of a population stewardship rights type of fishery system at the individual fisher level. In principle, EMUs could span the range from individual fishers to entire fishery sectors. Various characteristics may be used to define like-minded groups including fishing fleets, fisher associations, and geographic communities. In practice, the level of segregation or aggregation will be limited by the capability and commitment to monitoring the catches of identified EMUs as this will affect the precision of the required scientific advice as discussed above. With the currently available data for groundfish fisheries in Atlantic Canada, it may be more practical to begin with few EMUs and allow further fractionating when deemed desirable and feasible. A demand for high precision in the estimates of conceptual partial populations abundance for a large number of small EMUs may have to consider enhanced monitoring before implementation can be entertained. Resource users must balance the costs for increased monitoring associated with greater self-determination of small EMUs against the costs of negotiating common rules in larger EMUs.

Deliberation on the formula and mechanism for an initial sharing arrangement has proven contentious during the introduction of individual quota systems, but it should not be as problematic for population stewardship rights where the sharing arrangement only defines the starting point. Subsequent sharing of recruiting year-classes is based on the performance of EMUs as stewards of their conceptual partial populations. Unlike individual quota systems, in a population stewardship rights fishery, the EMUs have scope to change their fortune over time.

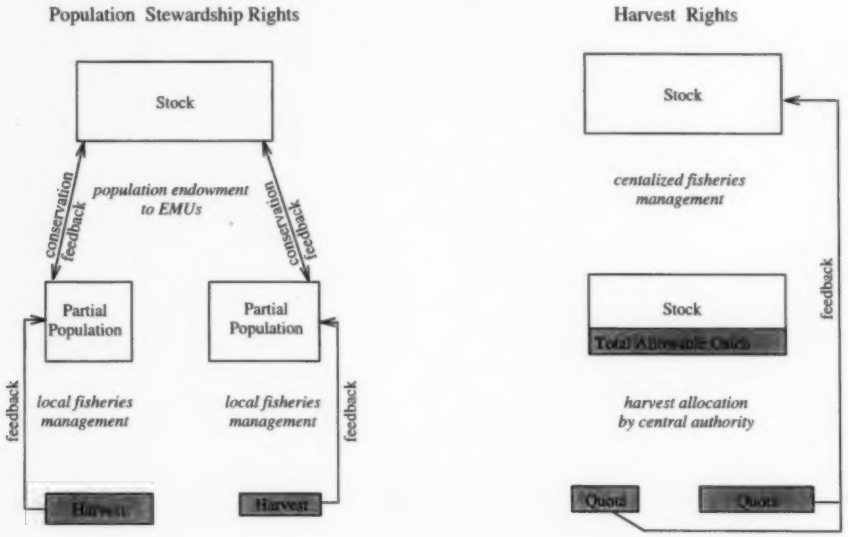
Responsible stewardship of the natural capital constituting a common property resource is connected to fairness in sharing of the benefits from its use. The perception that others are getting a free ride and are not held accountable for detrimental actions can disrupt orderly conduct (Ostrom 1990). The capability to partition the natural capital among EMUs makes it possible to grant authority and responsibility for management decisions but also provides a means to hold EMUs accountable for their actions (Fig. 4).

With harvest-rights systems like quota-based fisheries, a central authority determines stock-wide harvest levels, partitions that yield, and grants privileges for harvesting specified quantities among users. The impact of the harvest practices used by any user or group of users feeds back on the entire stock, affecting future total harvest. The benefits of restraint or the penalties of excess are dissipated among all participants; therefore, there is no incentive for any user to unilaterally take conservative actions.

With a population stewardship rights fishery system, the central authority partitions the stock into conceptual partial populations and contracts to the EMUs the responsibility to manage these population endowments. The consequences of each EMU's actions feed back on their own share of population. Although it is not possible to have complete isolation because of stock-wide dynamics, EMUs are effectively shielded from each other's actions by keeping track of the state of their conceptual partial population and ensuring that removals made by them are counted against their own assets. Knowledge of fisheries dynamics is employed to integrate a feedback

© 1996 NRC Canada

Fig. 4. The population stewardship rights fishery system partitions the stock natural capital among EMUs and uses established fishery dynamics to provide feedback on the consequences of their respective harvest activities. EMUs are effectively shielded from the actions of others by accounting for removals from the respective conceptual partial populations and allocating new recruits in accordance with the relative magnitudes of those partial populations. In contrast, for harvest-rights systems such as competitive or individual-quota fisheries, the actions of users affect the total stock and the consequences are dissipated among all.



system based on the value of natural capital and production from it, thereby reinforcing responsible stewardship and facilitating informed local decision making. Accountability is appropriately placed at that same local scale and participants derive motivation for sustainable practices by benefiting or suffering the consequences of their own actions. Those who persist with poor stewardship practices and fail to fulfill their contractual obligation to manage effectively can eventually deplete their respective population endowment and forfeit their privilege to participate in the fishery. Participants must be satisfied, though, that an acceptable level of confidence in fishery monitoring is achieved; otherwise, they may choose to fish out rather than bank their fish to prevent loss to others.

The possibility, however remote, that all EMUs could act inappropriately and irreversibly damage the productive capacity of the resource raises a legitimate public concern. It is reasonable to expect government to represent the interests of future generations. Where the recruitment processes are well understood, it should be possible for EMUs to craft conservation rules that meet with the approval of government. Practical rules for reserving portions of the population to reduce the risk of recruitment overfishing can be implemented, even when convincing relationships between spawning stock and recruitment have not been demonstrated. Actions by EMUs that result in maintenance of an adequate adult stock biomass could turn off or limit the safety reserve mechanism, thereby promoting conservative practices that consider longer time horizons.

By emphasizing accountability for actions as the means to motivate desired behavior, a population stewardship rights fishery system is vulnerable to events that undermine expected dividends. Numerous unpredictable factors, which are beyond the control of humans, influence fisheries productivity. EMUs that have dutifully exercised responsible stewardship only to see resources dwindle in response to external forces may question the virtue of their restraint. In a population stewardship rights fishery, as in most others, the impacts of these unpredictable events are borne proportionately by all. The potential for uncontrollable detrimental events to occur cannot be used as justification to avoid managing responsibly. These circumstances may orient some EMUs to consider risk-prone strategies whereas others may follow a more risk-averse approach, but they are still accountable for their actions. Recognizing the complexity of fisheries ecosystems, stability should not be expected and EMUs should be encouraged to develop fisheries that are robust to fluctuations in fish abundance.

Another potentially damaging externality is unpredictable government intervention. Governments can and should legitimately exercise their role in accordance with established and accepted rules. Adjustment and modification of rules, however, in response to lobbying by interest groups would undermine incentives to consider sustainable strategies over a long time horizon. Such political influences are incompatible with the establishment of enduring institutions for local governance. Changes to rules are preferably introduced by the local governing institutions, though there may be conservation conditions imposed by the central government, which the rules must satisfy.

In principle, centralized management offers great scope for public involvement in matters of resource utilization. Generally, however, it is impractical to keep the general public informed on detailed issues and to solicit their views frequently. Therefore, decision making is often delegated to an expert bureaucracy, generally a government department. Resource users who are most directly affected by that department's

© 1996 NRC Canada

management decisions often feel excluded by this arrangement, even when consultative processes are established. With externally imposed rules, adequate compliance often requires extensive central enforcement, which can be costly to implement effectively. A decentralized system can capitalize on the knowledge and social capital available in local communities. Sociologists consider that regulations that are devised and enforced with the participation of fishers are more likely to be effective (Pinkerton and Weinstein 1994). The heightened importance of local stewardship has the potential to encourage self-monitoring. Increased economic benefits from properly managed fisheries may prompt users to invest more resources towards resource monitoring to reduce uncertainties or even towards resource or habitat enhancement if this is practicable.

Common pool resources (Ostrom 1990), such as groundfish stocks in Atlantic Canada, which are difficult to manage under simple arrangements because of geographic scale, resource mobility, and diverse user base, pose the greatest challenge for increased participation by users in the management process. By recognizing the value of fish remaining in the water, by partitioning that value and providing flexibility to establish governing institutions at a scale smaller than a stock where many issues can be dealt with effectively and efficiently, and by incorporating feedback and accountability from actions that affect the vital fishery dynamic processes of recruitment, growth, and mortality, the population stewardship rights fishery system opens some avenues for increased fisher participation in local management of common pool resources.

Acknowledgments

I thank R.L. Stephenson, J.D. Neilson, E. Pinkerton, and two anonymous reviewers for their constructive criticisms. J.-J. Maguire, A. Sinclair, and R. Halliday provided helpful comments on an earlier version of this work. Finally, my appreciation goes to those fishers who gave their time to discuss these matters with me.

References

Anderson, T.L., and Leal, D.R. 1991. Free market environmentalism. Pacific Research Institute for Public Policy, San Francisco, Calif.

Angel, J.R., Burke, D.L., O'Boyle, R.N., Peacock, F.G., Sinclair, M., and Zwanenburg, K.C.T. 1994. Report of the workshop on Scotia–Fundy groundfish management from 1977 to 1993. Can. Tech. Rep. Fish. Aquat. Sci. No. 1979.

Canadian Atlantic Fisheries Scientific Advisory Committee. 1993. Annual report (including advisory documents). Vol. 15. Canadian Atlantic Fisheries Scientific Advisory Committee, Dartmouth, N.S.

Beverton, R.J.H., and Holt, S.J. 1957. On the dynamics of exploited fish populations. Reprinted 1993. Chapman & Hall, London.

Daly, H.E., and Cobb, J.B., Jr. 1989. For the common good: redirecting the economy toward community, the environment and a sustainable future. Beacon Press, Boston, Mass.

Gavaris, S. 1993. Assessment of the southwest Scotian Shelf and Bay of Fundy cod. DFO Atlantic fisheries research document No. 93/32. Department of Fisheries and Oceans, Ottawa, Ont.

Mace, P.M. 1994. Relationships between common biological reference points used as thresholds and targets of fisheries management strategies. Can. J. Fish. Aquat. Sci. **51**: 110–122.

Myers, R.A., Rosenberg, A.A., Mace, P.M., Barrowman, N., and Restrepo, V.R. 1994. In search of thresholds for recruitment overfishing. ICES J. Mar. Sci. **51**: 191–205.

Ostrom, E. 1990. Governing the commons: the evolution of institutions for collective action. Cambridge University Press, Cambridge, U.K.

Pinkerton, E., and Weinstein, M. 1995. Fisheries that work: sustainability through community-based management. David Suzuki Foundation, Vancouver, B.C.

Rees, W.E. 1990. The ecology of sustainable development. Ecologist, **20**: 18–23.

Rees, W.E. 1991. Conserving natural capital: the key to sustainable landscapes. Int. J. Can. Stud. **4**: 7–27.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Rivard, D., McKone, W.D., and Elner, R.W. 1988. Resource prospects for Canada's Atlantic fisheries, 1989–1993. Department of Fisheries and Oceans, Ottawa, Ont.

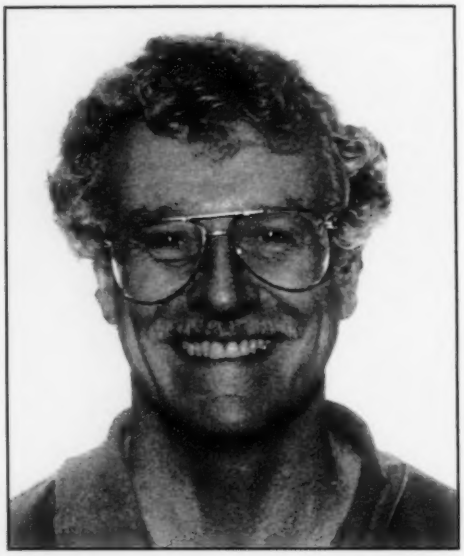
Townsend, R.E. 1992. Bankable individual transferable quotas. Mar. Policy, **16**: 345–348.

Townsend, R.E. 1995. Transferable dynamic stock rights. Mar. Policy, **19**: 153–158.

© 1996 NRC Canada

OBITUARY/NÉCROLOGIE

Robert Henry Peters
1946–1996



Rob Peters died in Montreal on 26 June 1996 at the age of 49. His death is a major blow to limnology and ecology.

In 1974 he joined the Department of Biology of McGill University and its Limnology Research Centre, after he obtained a B.Sc. and Ph.D. from the University of Toronto and held a postdoctoral fellowship in Europe. Rob did the work for his Ph.D. on phosphorus excretion in zooplankton under the supervision of Frank Rigler, an outstanding limnologist–ecologist who at the time was rethinking his scientific goals. Rigler's ideas deeply influenced Rob (see Peters 1983*a*) and in 1995 he published a book entitled *Science and Limnology*, originally meant to present Rigler's unpublished notes and lectures to a larger audience, but in reality an amalgam of their shared ideas for a more effective limnology and ecology.

During his postdoctoral work Rob met his future wife and fellow limnologist, Antonella Cattaneo, and through her acquired a close Italian family as well as a lifelong interest in Italian wines and good food. Parties at Rob and Nella's were renowned for the quantity and quality of primarily Italian wine and cheeses as well as pasta dishes. Particularly during the earlier years his house was one of the venues for sometimes heated discussions among faculty from the ecology group, and their graduate students, about how ecology is best practiced. These discussions, with Rob in the lead, have had a major influence on how work in limnology (and even evolutionary biology) is carried out at McGill and increasingly elsewhere as well.

Attracting Rob to McGill was a great stroke of good luck. He was an intellectual leader and a senior participant in the Lake Memphermagog Project, which grew into the present Limnology Research Centre. He subsequently played an important role in the successful establishment of the Groupe de recherche interuniversitaire en limnologie et environnement aquatique (GRIL), which unites limnologists from three Montreal universities.

Rob was an honest and delightfully modest man with a self-deprecating sense of humour, totally unaffected by any recognition that came his way. If anything, he minimized his accomplishments and rarely raised his voice. In contrast, as a critic he expressed his ideas in print forcefully, backed by the quantitative evidence he could assemble, about the unnecessary ineffectiveness of fundamental ecology and limnology, followed by proposed solutions to the problems. As he put it in a recent letter, "In my research, I have become obsessed with the inability of Ecology to provide more than the lore of natural history that I enjoyed as a child, or even to recognize the deficiencies. I have therefore set out [with others] to build a predictive ecology as a major contribution to a scientific revolution." Elsewhere he wrote, "Predictive limnology attracts those who are willing to sacrifice descriptive precision and detail for generality and application in predictions" (Peters 1986).

Rob Peters was deeply concerned about what he saw as (*i*) the ineffective contribution of academic ecology to the solving of environmental problems, (*ii*) the attention accorded to untested and frequently untestable notions, including the frequent uncritical acceptance of tautologies (Peters 1976), (*iii*) the paucity of hypotheses phrased in such a way that the chance that they can be refuted is maximized, and (*iv*) the only modest interest in predictive success as the ultimate measure of accomplishment.

An important underpinning for the work of many ecologists and biological limnologists is the conviction that it will be possible, in due course, to assemble the large number of components of the ecological pie into a comprehensive and comprehensible picture of the structure and functioning of whole systems. Rob questioned this belief. "Traditionally limnological questions are phrased in terms of the behaviour of individual species under intensive study in the laboratory or a particular water body. Descriptions of this behaviour are often complex, discursive, explanatory idealizations. They frequently transpose badly to other systems because the observed behaviour depends on the specific conditions of observation. Although a coherent view of the whole system might emerge from the aggregate of such studies, evidence in favor of this belief is not encouraging. Instead, success in traditional limnology is achieved by elaborating explanations that are consistent with widely held biological concepts. Thus, traditional

© 1996 NRC Canada

approaches serve to confirm and refine the received view of the external world" (Peters 1986). He then went on to confront the above approach with examples showing the power of a science geared to making useful predictions (Peters 1986).

Not surprisingly, Rob was severely criticized or dismissed by those who felt that he questioned the approach and utility of their work. Nor was his emphasis on the importance of predictive power as the ultimate measure of success much appreciated by those primarily fascinated by the identification of the mechanisms available to species, in causality, or in "understanding." They would question that "predictive power is the touchstone of science, is the ultimate criterion against which every scientific hypothesis can be tested" (Peters 1980*b*).

The few quotations presented cannot do justice to his extensively elaborated and, where feasible, documented arguments. His 1991 book *A Critique for Ecology* synthesizes his concerns and proposes solutions to what he (and not he alone) perceived to be the impediments that hinder the development of ecology and the contribution it makes both to the advancement of science and the solution of environmental problems.

Rightly or wrongly, most practising scientists pay little attention to the view of philosophers (of science), who often either are not scientists or lack much scientific credibility. Rob's critical views are not so easily dismissed because he was an outstanding limnologist with an international reputation, the author or co-author of about 100 published papers and four books. He had begun work on a fifth book entitled *Predictive Ecology*.

As his first major contribution to a more predictive ecology of greater utility he wrote *The Ecological Implications of Body Size* (Peters 1983*b*), a widely cited book. He saw the book as a practical and empirical tool to allow ecologists to make at least rough predictions on the basis of body size, in the absence of sufficient information for doing this on the basis of individual species in nature. The use of body size as a predictor was only one of three avenues of research that Rob pursued in his quest for a more useful ecology and limnology. The other two were (*i*) the utility of phosphorus in allowing predictions to be made about the abundance and distribution of plants and animals in lakes, estuaries, and oceans, and (*ii*) the possibilities afforded by the structure of organic chemicals for making predictions about their effects on the aquatic biota.

Rob's work is receiving considerable attention. A quantitative measure of this impact is the number of citations to his work by others. These numbered 267 and 293 in 1994 and 1995, an apparently exceptionally high number for ecology and limnology. For more on the use of citations see Peters et al. (1996). His qualities were recognized by the American Society of Limnology and Oceanography, which he served as a member at large at the time of his death, the North American Lake Management Society, for which he was Director of the Central Canadian Region in 1991–1993, and the Society of Canadian Limnologists, of which he was President this year. In 1991 he was awarded the Prize in Limnetic Ecology by the Ecology Institute of Oldendorf, Germany. He was admired, both as a scientist and person, by students and colleagues at McGill University and beyond. He was an excellent and much-liked supervisor of graduate students and an outstanding editor of their publications. His two upper-level courses in predictive ecology and aquatic management nicely complemented the more traditional offerings available to undergraduates. Rob is greatly missed but his contributions will have a long-term impact on limnology and ecology.[1]

Jacob Kalff
Department of Biology
Limnology Research Centre
McGill University
Montreal, QC H3A 1B1

Selected publications

Cyr, H., and Peters, R.H. 1996. Biomass-size spectra and the prediction of fish biomass in lakes. Can. J. Fish. Aquat. Sci. **53**: 994–1006.

Del Giorgio, P., and Peters, R. 1993. Balance between phytoplankton production and plankton respiration in lakes. Can. J. Fish. Aquat. Sci. **50**: 282–289.

Håkanson, L., and Peters, R.H. 1995. Predictive limnology. SPB Academic Publishing, The Hague, The Netherlands.

Hanson, J.M., and Peters, R.H. 1984. Empirical prediction of zooplankton and profundal macro benthos biomass in lakes. Can. J. Fish. Aquat. Sci. **41**: 439–445.

MacKenzie, B.R., Leggett, W.C., and Peters, R.H. 1990. Estimating larval fish ingestion rates: can laboratory derived values be reliably extrapolated to the wild? Mar. Ecol. Prog. Ser. **67**: 209–225.

Mailhot, H., and Peters, R.H. 1988. Relationships between 1-octanol/water partition coefficient and nine physico-chemical properties. Environ. Sci. Technol. **22**: 1479–1488.

Morin, A., Back, C., Chalifour, A., Boisvert, J., and Peters, R.H. 1988. Effect of black fly ingestion and assimilation on seston transport in a Quebec lake outlet. Can. J. Fish. Aquat. Sci. **45**: 705–714.

Peters, R.H. 1976. Tautology in evolution and ecology. Am. Nat. **110**: 1–12.

Peters, R.H. 1980*a*. From natural history to ecology. Perspect. Biol. Med. **23**: 191–203.

Peters, R.H. 1980*b*. Useful concepts for predictive ecology. Synthèse, **43**: 257–269.

Peters, R.H. 1983*a*. Frank Harold Rigler 1928–1982. Can. J. Fish. Aquat. Sci. **40**: 252. (Obituary.)

Peters, R.H. 1983*b*. The ecological implications of body size. Cambridge University Press, Cambridge, U.K.

Peters, R.H. 1984. Methods for the measurement of feeding, filtering and assimilation rates by zooplankton. *In* IBP Handbook No. 17. 2nd ed. *Edited by* J.A. Downing and F.H. Rigler. Blackwell Scientific Publications Ltd., Oxford, U.K.

Peters, R.H. 1986. The role of prediction in limnology. Limnol. Oceanogr. **31**: 1143–1159.

Peters, R.H. 1991. A critique for ecology. Cambridge University Press, Cambridge, U.K.

Peters, R.H., and Downing, J.A. 1984. Empirical analysis of zooplankton filtering and feeding rates. Limnol. Oceanogr. **29**: 763–784.

Peters, R.H., and Raelson, J.V. 1984. Relations between individual size and mammalian population density. Am. Nat. **124**: 498–517.

Peters, R.H., and Rigler, F.H. 1973. Phosphorus release by *Daphnia*. Limnol. Oceanogr. **18**: 821–839.

[1] A scholarship fund in Rob's name is being established. Donations, which are tax deductible, can be made out to McGill University – R.H. Peters Scholarship, and forwarded to Kathryn Peterson, Administrative Officer, Department of Biology, McGill University, 1205 Dr. Penfield Avenue, Montreal, QC H3A 1B1, Canada.

© 1996 NRC Canada

Peters, R.H., and Wassenberg, K. 1983. The effect of size on animal abundance. Oecologia, **60**: 89–96.

Peters, R.H., Ball, G.E., Carignan, R., Hebert, P.D.N., and Prepas, E.E. 1996. An assessment of research in evolution and ecology supported by the Natural Sciences and Engineering Research Council of Canada. Can. J. Fish. Aquat. Sci. **53**: 670–680.

Rigler, F.H., and Peters, R.H. 1995. Science and limnology. Ecology Institute, Oldendorf–Luhe, Germany.

Shipley, B., and Peters, R.H. 1990. A test of the Tilman model of plant strategies: relative growth rate and biomass partitioning. Am. Nat. **136**: 139–153.

Tang, E., and Peters, R.H. 1995. The allometry of algal respiration. J. Plankton Res. **17**: 303–315.

© 1996 NRC Canada

THE SOCIETY FOR CONSERVATION BIOLOGY ANNUAL MEETING

June 6 to 9, 1997
Victoria, British Columbia, Canada

The 1997 SCB Annual Meeting offers an opportunity to present papers and posters on a variety of topics, with a major theme this year on **Marine Conservation Biology**. Invited and contributed special sessions will be held concurrently with other non-marine sessions, but the special emphasis in a number of symposium sessions will be similarities and differences between conservation biology in the marine setting and in other realms. Oral and poster sessions will be scheduled over three days, with evening times available for roundtables and discussion groups. Single and multi-day field trips are planned for the June 10 - 14 period, and will include topics and destinations such as: Forests and Forestry in the Carmanah Valley, Gray Whales of Clayoquot Sound, Marine Biota of the Strait of Georgia, and Ethnobotany of British Columbia Aboriginal People.

Call for Papers and Posters deadline is January 15, 1997. For author instructions and information on abstract submissions, please refer to the conference web site at http://geography.geog.uvic.ca/dept/announce/scb_page.html.

Program Information: Dr. David Duffus, Dept. of Geography, University of Victoria, PO Box 3050, Victoria, BC, Canada, V8W 3P5. Phone (250) 721-7344, FAX (250) 721-6216, Email dduffus@office.geog.uvic.ca.

Conference Coordination: Pat McGuire, Conference Management, University of Victoria, PO Box 3030, Victoria, BC, Canada, V8W 3N6. Phone (250)721-8746, FAX (250)721-8774, Email SCB97@uvcs.uvic.ca.

SUBSCRIBE TODAY

NRC RESEARCH PRESS

Subscription Office, M-55
NRC Research Press
National Research Council Canada
Ottawa, ON K1A 0R6
Canada
Tel.: 613-993-9084
Fax.: 613-952-7656
E-mail: research.journals@nrc.ca
URL: http://www.nrc.ca/cisti/journals/rj.html

ABONNEZ-VOUS AUJOURD-HUI

PRESS SCIENTIFIQUES DU CNRC

Bureau d'abonnements, M-55
Presses scientifiques du CNRC
Conseil national de recherches Canada
Ottawa, ON K1A 0R6
Canada
Tél. : 613-993-9084
Télécopieur : 613-952-7656
Courrier éléctronique : research.journals@nrc.ca
URL : http://www.nrc.ca/cisti/journals/rj.htm

CANADIAN CONFERENCE FOR FISHERIES RESEARCH (CCFFR)

Hotel Radisson
Ottawa, Ontario
2-4 January 1997

The Golden Jubilee meeting of the Canadian Conference for Fisheries Research (CCFFR) is scheduled for 2-4 January 1997 in Ottawa. CCFFR, which meets in concert with the Society of Canadian Limnologists (SCL), is designed as a forum where Canadian researchers and graduate students can discuss the latest advances in freshwater and marine fisheries research.

The major themes of the 50th Anniversary Conference will be:

1) Stock/population structure and dynamic processes for informed decision making (e.g. multi-metrics; phenotypic/genotypic studies; distribution and migration; mortality and recruitment)

2) Ecophysiology and environmental processes: influences on temporal and spatial states of individuals and populations

3) Quantifying the effects of fishing on aquatic populations and their habitats; direct effects and the utility of fisher and fleet information

4) **Joint CCFFR/SCL Session:**

   Techniques and technology in aquatic sciences: standards, advances, and expectations

5) **Special Symposium Session:**

   Recent advances in Atlantic cod research. This session will focus on recent cod research that falls under the themes outlined above (e.g. physiology, condition, mortality, recruitment, predator-prey, ecosystem dynamics etc.)

Papers addressing the major themes are encouraged, particularly as they relate to using knowledge to solve pressing problems in conservation and exploitation. Submissions on other themes related to fisheries and aquatic habitat biology are also welcome.

CONFERENCE CANADIENNE DE LA RECHERCHE SUR LES PECHES (CCRP)

Hotel Radisson
Ottawa (Ontario)
les 2-4 janvier 1997

Le Jubilé de la Conférence canadienne de la recherche sur les pêches (CCRP) se tiendra du 2 au 4 janvier à Ottawa. La CCRP, qui se réunit de concert avec la Société canadienne des limnologistes (SCL), se veut un forum où les chercheurs et étudiant/es gradué/es canadien/nes peuvent discuter des derniers progrès de la recherche halieutique en eaux douces et eaux marines.

Les principaux thèmes abordés lors de la Conférence du 50e Anniversaire seront:

1) Structures et processus dynamiques des stocks/populations permettant une prise de décision raisonnée (approches multimétriques, études phénotypiques ou génotypiques, distribution, migrations, mortalité et recrutement)

2) Ecophysiologie et processus environne mentaux: leurs influences sur les caractéristiques temporelles et spatiales des individus et des populations

3) Quantifier les effets de la pêche sur les populations aquatiques et leurs habitats; effets directs et l'utilité d'informations provenant des pêcheurs et des flottes

4) **Session Conjointe CCRP/SCL:**

   Les techniques et la technologie dans les sciences aquatiques : standards, progrès, attentes

5) **Symposium spécial:**

   Progrès récents dans la recherche sur la moru franche. On traitera des recherches récentes reliées aux thèmes décrits ci-haut (physiologie, condition, mortalité, recrutement, relations prédateur-proie, dynamiques des écosystèmes, etc)

Des contributions reliées aux thèmes principaux sont particulièrement sollicitées, surtout celles qui discutent de l'utilisation des connaissances pour resoudre les problèmes importants de conservation ou d'exploitation. Des contributions sur d'autres thèmes reliés aux sciences halieutiques et à la biologie des habitats aquatiques sont également les bienvenues.

Program Chairman / Président du Programme

Dr. C.T. Taggart
Oceanography Department
Dalhousie University
Halifax, NS B3H 4J1

tel: 902-494-7144 fax: 902-494-3877 internet: chris.taggart@dal.ca
Updated information – http://www.phys.ocean.dal.ca/ccffr/index.html – Informations à jour

Department of Fisheries and Oceans
Green Plan Toxic Chemicals Program
Wrap-up Conference

January 28 - 31, 1997
Government Conference Centre
Ottawa, Ontario

In recent years, major advances have been made in our understanding of the fate of toxic chemicals in aquatic ecosystems and their effects on fish and fish habitat.

A conference will be held to: (1) present the results of the research funded over the last five years of the DFO Green Plan Toxic Chemical Program; and (2) provide a forum for the exchange of information and the discussion of critical current and future toxic chemical issues.

Over 80 research papers will be presented in oral and poster formats.

The Conference's three main themes are:

- ☐ Fate in aquatic ecosystems;
- ☐ Effects ranging from the biochemical/cellular to the whole organism/ecosystem level; and
- ☐ Chemical and biological measurement methodologies.

No registration fees will be charged to attend this event, but attendance is limited.

For more information please contact:
Darren Williams, c/o Department of Fisheries and Oceans, Station 1280, 200 Kent St., Ottawa, Ontario, Canada, K1A 0E6. Tel: (613) 834-3065; Fax: (613) 834-2858 or (613) 990-5510; Darren.Williams@ncr.ottwpo.dmpo.x400.gc.ca

Conférence de synthèse sur le Programme des chimiques toxiques du Plan vert du ministère des Pêches et des Océans

Du 28 au 31 janvier 1997
Centre de conférences du gouvernement
Ottawa (Ontario)

Depuis quelques années, nous avons fait d'importants progrès dans notre compréhension du devenir des chimiques toxiques dans les écosystèmes aquatiques, et de leurs effets sur les poissons et les habitats.

La conférence a pour objet : (1) de présenter les résultats de la recherche financée au cours des cinq dernières années dans le cadre du Programme des chimiques toxiques du Plan vert du MPO; et (2) de servir de forum permettant d'échanger des renseignements et de discuter des problèmes fondamentaux, actuels et futurs, liés aux chimiques toxiques.

Plus de 80 rapports de recherche seront présentés oralement et sous forme d'affiche.

Les trois principaux thèmes de la conférence sont :

- ☐ le devenir des chimiques toxiques dans les écosystèmes aquatiques;
- ☐ toute la gamme des effets des chimiques toxiques: du niveau biochimique/cellulaire au niveau de l'organisme/écosystème en entier; et
- ☐ les méthodes de cueillette des données chimiques et biologiques.

Il n'y a pas de frais d'inscription, cependant, les places sont limitées.

Pour de plus amples renseignements, veuillez communiquer avec: Darren Williams a/s du ministère des Pêches et des Océans, poste 1280, 200, rue Kent, Ottawa (Ontario) K1A 0E6; Tél. : (613) 834-3065; Fax : (613) 834-2858 ou (613) 990-5510; Courrier électronique : darren.williams@ncr.ottwpo.dmpo.x400.gc.ca

OBITUARY NÉCROLOGIE

1692–1694 Robert Henry Peters

Thomas P. Quinn and N. Phil Peterson	**1555–1564**	The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef Creek, Washington
Andreas Winter, Jan J.H. Ciborowski, and Trefor B. Reynoldson	**1565–1571**	Effects of chronic hypoxia and reduced temperature on survival and growth of burrowing mayflies, *Hexagenia limbata* (Ephemeroptera: Ephemeridae)
J.E. Paloheimo and Y. Chen	**1572–1579**	Estimating fish mortalities and cohort sizes
Susan E. Wilson, Brian F. Cumming, and John P. Smol	**1580–1594**	Assessing the reliability of salinity inference models from diatom assemblages: an examination of a 219-lake data set from western North America
Peter M. Kiffney	**1595–1601**	Main and interactive effects of invertebrate density, predation, and metals on a Rocky Mountain stream macroinvertebrate community
D.R. Marmorek, R.M. MacQueen, C.H.R. Wedeles, J. Korman, P.J. Blancher, and D.K. McNicol	**1602–1608**	Improving pH and alkalinity estimates for regional-scale acidification models: incorporation of dissolved organic carbon
Doran M. Mason and Stephen B. Brandt	**1609–1617**	Effect of alewife predation on survival of larval yellow perch in an embayment of Lake Ontario
G. Mertz and R.A. Myers	**1618–1625**	Influence of fecundity on recruitment variability of marine fish
R. Brent Summers, James H. Thorp, James E. Alexander, Jr., and Ronald D. Fell	**1626–1631**	Respiratory adjustment of dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) in response to chronic turbidity
J.Y. Wang, D.E. Gaskin, and B.N. White	**1632–1645**	Mitochondrial DNA analysis of harbour porpoise, *Phocoena phocoena*, subpopulations in North American waters
Lauri Arvola, Paula Kankaala, Tiina Tulonen, and Anne Ojala	**1646–1662**	Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake (Lake Pääjärvi)
Paula Kankaala, Lauri Arvola, Tiina Tulonen, and Anne Ojala	**1663–1674**	Carbon budget for the pelagic food web of the euphotic zone in a boreal lake (Lake Pääjärvi)
Yongshun Xiao	**1675–1682**	How does somatic growth rate affect otolith size in fishes?
PERSPECTIVE		PERSPECTIVE
Stratis Gavaris	**1683–1691**	Population stewardship rights: decentralized management through explicit accounting of the value of uncaught fish

National Research Council Canada
Ottawa, Canada K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada K1A 0R6

Canadian Journal of Fisheries and Aquatic Sciences

Volume 53, Number 7 July 1996

Journal canadien des sciences halieutiques et aquatiques

Volume 53, numéro 7, juillet 1996

ARTICLES		ARTICLES
K.T. Frank, J.E. Carscadden, and J.E. Simon	**1473–1486**	Recent excursions of capelin (*Mallotus villosus*) to the Scotian Shelf and Flemish Cap during anomalous hydrographic conditions
D.K. Hawkins and T.P. Quinn	**1487–1496**	Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids
Christos D. Maravelias, David G. Reid, E. John Simmonds, and John Haralabous	**1497–1505**	Spatial analysis and mapping of acoustic survey data in the presence of high local variability: geostatistical application to North Sea herring (*Clupea harengus*)
Josef Wanzenböck, Monika Zaunreiter, Christina M. Wahl, and David L.G. Noakes	**1506–1512**	Comparison of behavioural and morphological measures of visual resolution during ontogeny of roach (*Rutilus rutilus*) and yellow perch (*Perca flavescens*)
C. Hudon, R. Morin, J. Bunch, and R. Harland	**1513–1525**	Carbon and nutrient output from the Great Whale River (Hudson Bay) and a comparison with other rivers around Quebec
P.P. Pomeroy, N. Green, A.J. Hall, M. Walton, K. Jones, and J. Harwood	**1526–1534**	Congener-specific exposure of grey seal (*Halichoerus grypus*) pups to chlorinated biphenyls during lactation
Olof Regnell, Anders Tunlid, Göran Ewald, and Olof Sangfors	**1535–1545**	Methyl mercury production in freshwater microcosms affected by dissolved oxygen levels: role of cobalamin and microbial community composition
Jörgen I. Johnsson, Erik Petersson, Elisabeth Jönsson, Björn Th. Björnsson, and Torbjörn Järvi	**1546–1554**	Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, *Salmo trutta*

Continued on inside back cover / Suite au verso

Canada

Printed in Canada by / Imprimé au Canada par
University of Toronto Press Inc.
Typesetting by / Typographie par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(199607)53:7;1-X